ABSTRACT OF THESIS

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Title of Thesis Studies on the Sulphonyl Esters of Carbohydrates.

The general principles of conformational analysis in the sugar series have been reviewed briefly in the introduction.

The 6-tosyl esters of a number of glycopyranosides and some related compounds have been prepared by direct tosylation of the corresponding methyl glycoside or deoxy compound. A simple counter current extraction procedure was found to be a useful method of isolating the product in some cases.

The kinetics of the reaction between sodium hydroxide and a 6-tosyl ester, capable of ring closing to form a 3,6-anhydro compound, have been studied. Attempts to determine directly, the expected second-order rate constant for the reaction, gave results which did not obey the accepted second-order law. Further examination of the products of the reaction, revealed that those 3,6-anhydro sugars having a 1,3-diaxial arrangement of hydroxyl groups in their most probable conformation, were appreciably acidic. Infrared measurements on a number of 3,6-anhydro compounds showed that the acidic compounds contained a very strong intramolecular hydrogen bond in the un-ionised state.

The difficulties involved in the kinetic measurements were avoided by determining first-order rate constants using a large excess of sodium hydroxide for the reaction. Measurements were made at various concentrations of sodium hydroxide for each compound and a considerable increase in the derived second-order rate constant ($\frac{k_1}{[OH^-]}$) with increasing hydroxide ion concentration was observed with those compounds giving rise to the more acidic 3,6-anhydro compounds. A kinetic scheme is described in which the reaction is assumed to occur partly via a mono ionised species and partly via a di-ion, the latter is particularly important in those compounds in which a 1,3-diaxial arrangement of hydroxyl groups is possible and it is suggested that the di-ion is stabilised by hydrogen bonding.

Using the method of least squares, second-order rate constants have been derived for each compound. The rate constants so obtained have been discussed in terms of sugar conformations, and the importance of dipole interactions and hydrogen bonding in determining conformation, is pointed out.

STUDIES ON THE
SULPHONYL ESTERS OF CARBOHYDRATES.

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by

Ronald Baker.

Thesis presented for the degree of Doctor of Philosophy
at the University of Edinburgh.

1962.

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A C K N O W L E D G E M E N T

The author wishes to express his thanks to the Board of Directors of Imperial Chemical Industries, for providing the opportunity to do this work. To Professor E.L. Hirst, F.R.S. in whose laboratory the experiments were carried out, and to Dr. J.C.P. Schwarz, for constant advice and encouragement. Also to my sisters, Barbara and Mona, in appreciation of their work in typing this Thesis.

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INTRODUCTION

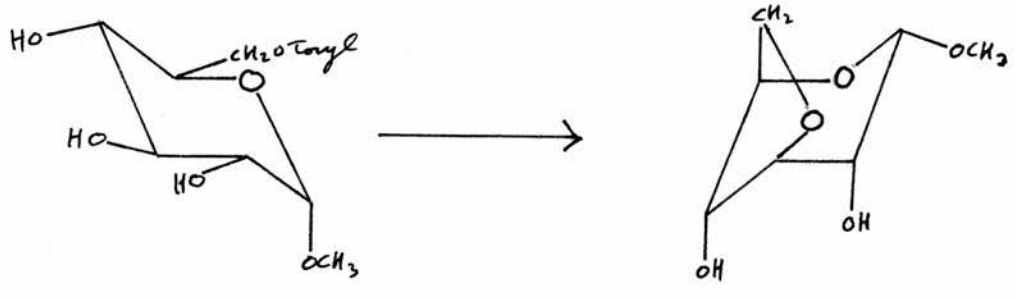
Because of their versatility in synthetic work much is known about the preparation and properties of the sulphonyl esters of sugar molecules and these compounds have been reviewed comprehensively by Tipson[1]. Of special value are the reactions of sulphonyl esters of carbohydrates with alkalis, and the formation of oxide rings by the action of alkali upon suitably orientated sulphonyl esters of carbohydrates is well known. Since in many cases the toluene-p-sulphonyl (=tosyl) esters are readily obtained in the crystalline form, they have been widely used in preparative work and the present discussion is limited to these compounds.

As long ago as 1912 Ferns and Lapworth[2] pointed out that whereas carboxylic esters of simple alcohols undergo acyl-oxygen fission in the presence of alkali, the esters of "strong acids" such as sulphuric, phosphoric or nitric acid, or of sulphonic acids, tend towards alkyl-oxygen fission. This is readily shown by the alkaline hydrolysis of the esters of an optically active compound; carboxylic esters are almost invariably hydrolysed with retention of configuration but for esters of "strong acids" inversion or racemisation often occurs. By analogy it follows that the formation of anhydro sugars from tosyl esters depends upon scission of the carbon-oxygen bond. A necessary condition for the production of anhydro rings is that in addition to the ester grouping a suitably orientated hydroxyl group or its precursor must also be present in the molecule. Where rotation is restricted as in the pyranose or

furanose derivatives of sugars, three-membered epoxide rings are formed only if the carbon atom adjacent to that bearing the tosyloxy group carries an hydroxyl group which bears a trans relationship to the ester group. Ring formation is thought to occur through intramolecular displacement of the sulphonyloxy group by the ion produced from the hydroxyl under the action of alkali and is invariably accompanied by inversion at the carbon atom carrying the ester grouping. The formation of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannoside from methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside[3] illustrates this point. The formation of epoxide rings in this way has proved of immense value in synthetic work, as opening the ring provides a ready method for the preparation of some of the less common sugars from the more accessible ones. If no suitable hydroxyl group is present, the sulphonic ester moiety is removed only with difficulty and configuration is retained. The chemistry of the sugar epoxides has been reviewed recently by Newth[4] and will not be considered further here.

Of primary importance to the present work is the formation of a 3,6-anhydro ring when the 6-tosyl ester of a suitable sugar is treated with alkali; this reaction has been investigated widely.

Methyl 3,6-anhydro-α or β-D-glucopyranoside is formed readily when the corresponding 6-tosyl ester of the glycoside is treated with alcoholic sodium hydroxide solution[5].



The 3,6-anhydro derivatives of galactose[6] and mannose[7] may be obtained in a similar manner. A consideration of the configurations of those 6-tosylates which give 3,6-anhydro-compounds reveals that the sulphonyloxy group and the hydroxyl group at $C_{(3)}$ bear a cis relationship, this condition being necessary for the intramolecular attack of $O^-_{(3)}$ on $C_{(6)}$. It may be noted here that the action of alkali on methyl 3-O-tosyl-α-D-glucoside leads to the formation of three-membered epoxide rings[8a] rather than the 3,6-anhydro compound as the main product, although traces of 3,6-compound are formed by epoxide migration[8b].

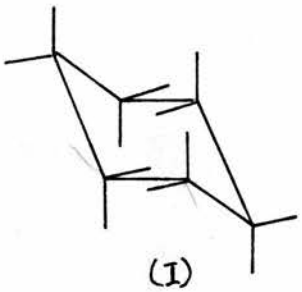
The main interest in the formation of 3,6-anhydro compounds from the point of view of the present work is a conformational one. In recent years conformational analysis has proved of immense value in the interpretation of many of the puzzling features of carbohydrate chemistry. Many excellent reviews have been published on this topic[4,9] and only a brief survey of some of the concepts and applications will be given here.

The conformation of pyranose sugars

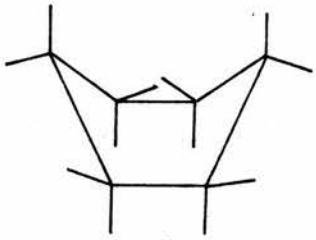
A great number of sugars and their derivatives exist in a cyclic form; of these, the six-membered or pyranose ring is the most common. For many years the projection formulae of Haworth[10] proved satisfactory for the representation of reactions in the sugar series; however, it became increasingly obvious that a more precise representation of the structures was necessary for a better understanding of the stereochemical problems of carbohydrate chemistry. In recent years the principles of conformational analysis[11] have been applied with considerable success.

The conformational aspect of the stereochemistry of cyclohexane and its

derivatives has been investigated extensively[12] and it is well established that whenever possible the molecule adopts one or other of the possible forms in which the carbon atoms in the ring exhibit their normal valency angle of (109.5°) and consequently the ring is non-planar. For cyclohexane two arrangements in space of the carbon atoms for which angle strain is a minimum have been shown to be of particular importance and these have been designated the "chair" (I) and "boat" (II) forms respectively.

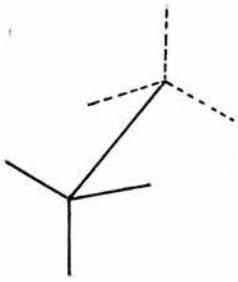


(I)

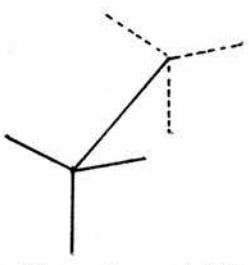


(II)

It will be seen that, of the twelve C-H bonds in the chair conformation, six lie parallel to the three-fold axis of symetry of the ring and are termed axial or '<u>a</u>'; the remaining six are directed radially and are termed equatorial or '<u>e</u>'. Further consideration of the chair and boat forms shows that in the former the hydrogen atoms all occupy staggered positions thus minimising non-bonded replusions whereas in the boat form some of the atoms are eclipsed. Particularly disadvantageous are interactions between the atoms at the "bow and stern flagpoles" of the boat.



staggered positions



eclipsed positions

As a consequence of this, non-bonded interactions are generally less in the chair conformation and whenever possible the chair conformation is preferred. In the case of substituted cyclohexanes, two chair conformations can be distinguished and it has been shown by a variety of methods that the conformation which places the majority of the bulkier substituents in equatorial positions is generally the more stable.

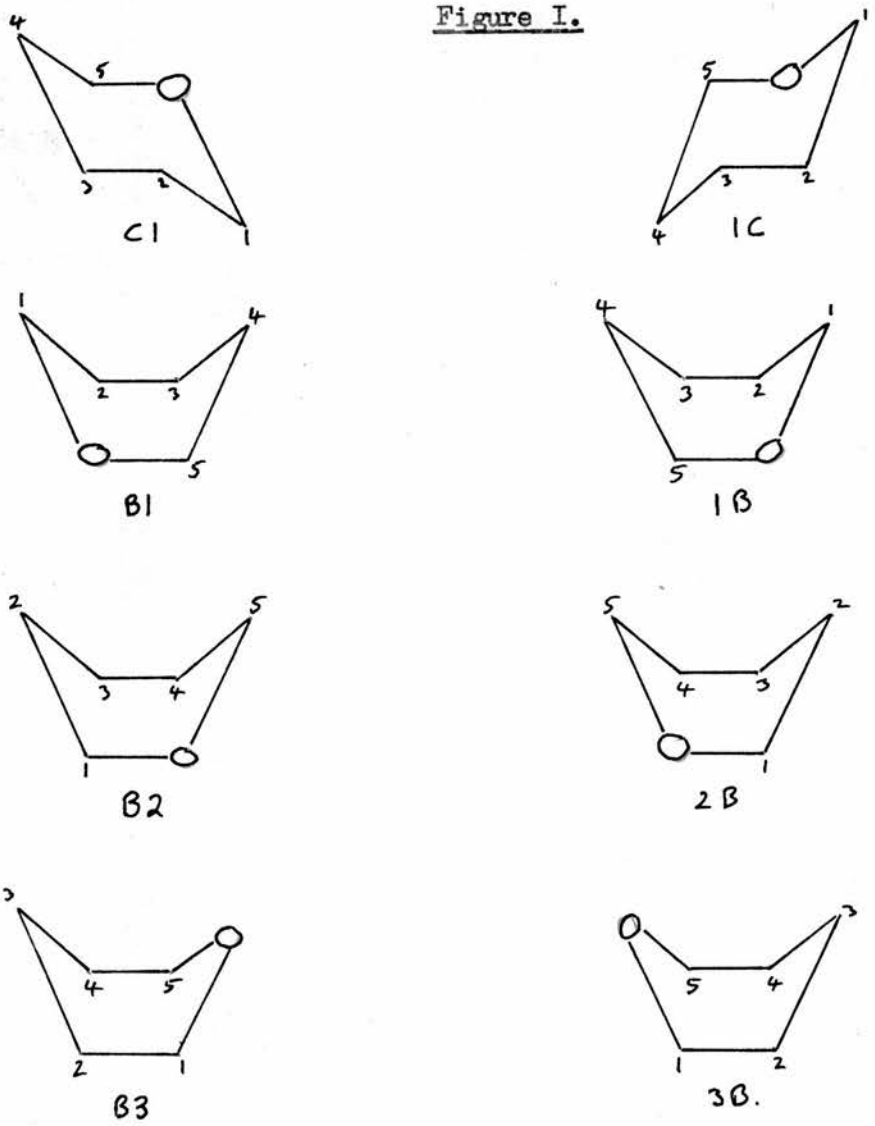
The pyranose skeleton of sugars can be regarded as cyclohexane in which one of the carbon atoms has been replaced by oxygen; this causes only minor changes in the bond angles within the ring[13] and the conformational principles of cyclohexane chemistry may be expected to apply. In addition to the two possible chair conformations of the pyranose ring, it is necessary to consider a number of possible alternatives based on the boat conformation. In his pioneering work, Reeves[14] illustrated a cycle consisting of six interconvertible boat conformations which were designated B1, 1B, B2, 2B, B3 and 3B. These are shown in Figure 1, together with the two chair conformations (C1 and 1C).

Recently it has been pointed out that the boat conformations are flexible and represent extremes of an infinite number of intermediate structures.[15] Of particular interest are those arrangements in which three ring atoms and the centre atom of those remaining are coplanar. This "skew" or "stretched" form is depicted in Figure 2, Reeves[16] has pointed out that one such arrangement occurs midway between each pair of the boat conformations shown in Figure 1. It is to be noted that whilst the non-bonded interactions in such arrangements are less than in any boat conformation they are still, in most molecules, in excess of those present in chair conformations. Although there is little

direct experimental evidence for the existence of skew conformations, it seems probable that in compounds in which both chair conformations involve serious repulsions between bulky axial substituents, the molecules will assume a shape intermediate between two boat conformations.

Another type of conformation, which must be considered for compounds in which unsaturation or the presence of fused rings requires that four adjacent atoms of the ring are coplanar, is a half chair form (Figure 3). Such structures are likely for glycals and for epoxide derivatives of pyranose sugars.

Figure I.



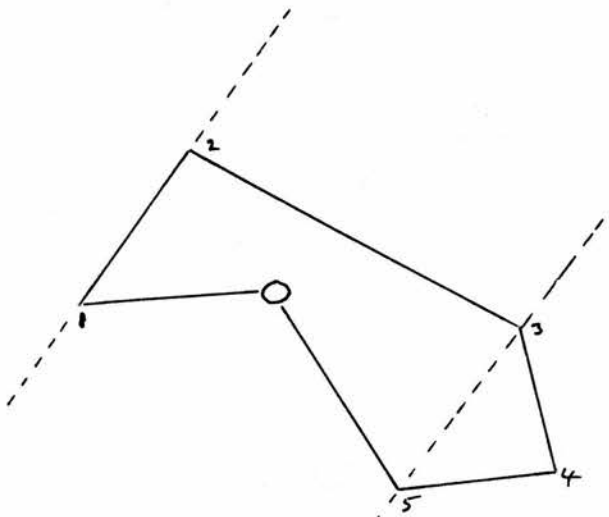


Figure 2.

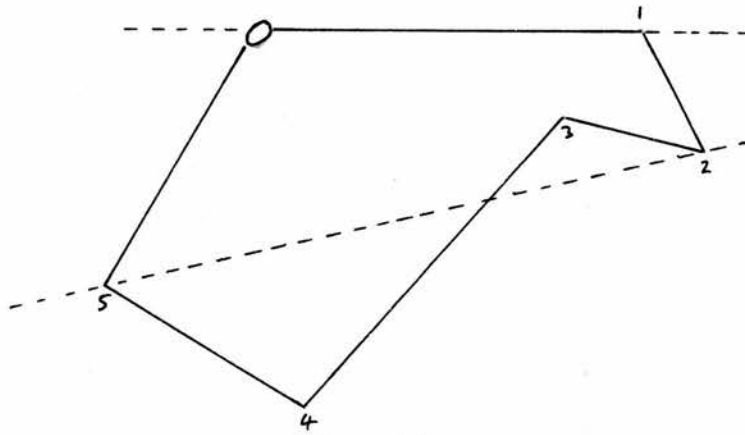


Figure 3.

Nomenclature

The unambiguous naming of conformers is difficult. The designations given above in Figure 1 have been widely used but they have the disadvantage that the conformation designated C1 in the D series of sugars becomes the 1C conformation in the L series. To avoid this, Guthrie[17] and Ishell and Tipson[18] have independently devised essentially the same system of nomenclature based on the orientation at the anomeric carbon atom. The two chair conformations are designated as CA or CE depending on whether the α-anomeric group is (or would be) axial or equatorial. The boat forms are named in a similar manner. Compounds now designated as CA, CE, B_1A, B_1E, B_2A, B_2E, B_3A, B_3E conformations have, in Reeves classification, C1, 1C, 1B, B1, B2, 2B, B3, 3B rings respectively when in the D series; when in the L series, they have 1C, C1, B1, 1B, 2B, B2, 3B and B3 rings respectively. It must be borne in mind that the symbols A and E do not, of themselves, mean that an axial or equatorial anomeric group is actually present in the molecule. A consideration of the conformation of methyl β-D-galactoside (Figure 4) which under the present scheme is denoted CA

serves to illustrate this point.

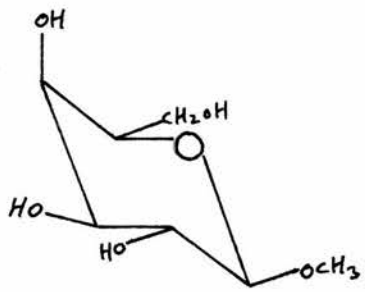


Figure 4.

Isbell and Tipson have also proposed nomenclature (based on the letter S) for the various skew forms.

So far as the present work is concerned the D configurations of hexoses only are involved and the original C1, 1C, B1, 1B, B2, 2B, B3, 3B conventions of Reeves have been retained.

Theoretical predictions and the experimental evidence for the existence of preferred conformations

On theoretical grounds Reeves concluded that whenever possible a chair conformation is preferred and that the conformation can in many cases be deduced from a consideration of the following "instability factors" :-

i) The stability of a conformation decreases with an increasing number of axial substituents other than hydrogen.

ii) An axial hydroxymethyl group at $C_{(5)}$ on the same side of the ring as any other bulky substituent is very unfavourable - "The Hassel-Ottar effect"[13].

iii) An axial hydroxyl group at $C_{(2)}$ the C-O bond of which bisects the angle between the two $C_{(1)}$-O bonds, leads to additional instability. This latter effect is probably largely due to dipolar interactions between the hydroxyl groups at $C_{(1)}$ and $C_{(2)}$ and the ring oxygen atom (see page 95).

As a result of extensive study[14] of the complexes which cuprammonium

solutions form with sugar molecules, Reeves, was able to confirm many of the predictions of conformation made on the above basis. Complex formation was detected by a decrease in the conductivity of the cuprammonium solution and in some cases by a very large change in optical rotation. Using model systems in which a 1:2 diol grouping was held in fixed relationship, Reeves found that a decrease in the conductivity of cuprammonium solutions occurred when the hydroxyl groups had a projected angle of 0°, as in the case of eclipsed groups, or of $\pm 60^{\circ}$, as represented by an axial-equatorial or equatorial-equatorial relationship. If the projected angle was greater than 60°, complex formation did not occur; the non-formation of complexes in certain cases was regarded as significant evidence for conformational purposes. Using suitably substituted derivatives of methyl β-D-glucopyranoside, Reeves demonstrated that a laevorotatory complex is formed by 2,3-diols, whereas the complex with 3,4-diols is dextrorotatory and that little or no complex formation occurs elsewhere in the molecule. For derivatives of methyl β-D-glucoside having free hydroxyl groups at $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$, the simultaneous formation of laevo- and dextrorotatory "compensating" complexes resulted in only small changes in rotation but a marked change in conductivity. Similar complexes were found to be formed in the D-galactopyranoside series except that in this case laevorotatory complexes are formed at both the 2,3 and 3,4 positions. In both cases the results are in accord with the prediction that these glycosides exist in the C1 conformation.

An analysis of a range of methyl glycopyranosides using the above technique showed excellent correlation between the conformations deduced on the basis of complex formation and those predicted from a consideration of the

instability factors discussed previously. Especially relevant to the present work are the C1 conformations assigned to the methyl α and β-glycopyranosides of the glucose and galactose series.

Reeves[19] observed the formation of one other type of complex with cuprammonium solutions. 1,6-Anhydro-β-D-glucopyranose and its 3-methyl derivative, which on theoretical grounds are believed to exist in the 1C conformation[13] showed some evidence of the formation of low rotating complexes involving the hydroxyl groups on carbon atoms 2 and 4. This provides some confirmation of the 1C conformation since an approximate calculation shows that the $O_{(2)}$-$O_{(4)}$ distance is within the limit for complex formation. A similar type of complex has been encountered with methyl 3-O-methyl-β-D-idopyranoside.

While a general picture of carbohydrate conformations has emerged from this and other work, attempts to formulate more precise rules for the quantative prediction of the relative stability of various conformations meet with great difficulties; each molecule presents unique features which must be considered. The effect of the non-bonded interactions between the substituents is in many cases modified by circumstance; thus, for example, it is in no way certain that the conformation which prevails in aqueous solution will be the same in anhydrous solvents or in the solid state.

One weakness of the cuprammonium method is that changes in conformation may occur as a result of complex formation if the energy difference between the conformations is small (this criticism does not apply to cases where the non-formation of complexes is significant). However, the conformational assignments made by Reeves have been largely confirmed by a number of other

methods, the most important of which are briefly discussed below. Physical methods, especially nuclear magnetic resonance, are of particular value in this connection, since they eliminate the criticism of chemical methods mentioned above; namely, that the energy of reaction may in some cases be sufficient to bring about changes in conformation.

The nuclear magnetic resonance spectra of carbohydrates provide information about conformation in two distinct ways[20] In the first place, it has been found that equatorial anomeric protons absorb at lower fields than axial anomeric protons. Secondly the magnitude of the spin-spin coupling between adjacent protons is much greater if the projected angle between the C-H bonds is 180° (both axial) than when it is 60° (ea or ee arrangements). The acetylated aldopyranoses have been studied in detail and in most cases the spectra provide direct evidence that these compounds exist in the chair conformation which has the least number of substituents, other than hydrogen, in axial positions.

X-ray analysis by Beevers and Mc'Donald[21] has shown that the pyranose ring in crystalline α-D-glucose has the C1 conformation. The work also established the presence of a cis diol grouping at carbon atoms one and two, providing direct evidence for the usually accepted configuration at the anomeric centre for α-D-glucose. Work on α-D-rhamnose monohydrate[22] and sucrose sodium bromide dihydrate[23] also showed the pyranose ring to be in the C1 form. Useful evidence was obtained in the latter case for the shape of the furanose ring, previously regarded as being essentially planar. Analysis showed the carbon atoms one, two, three and the ring oxygen to be in one plane with carbon atom four distant $\frac{1}{2}$Å from the plane. The conformations of five-membered

rings have also been studied in solution using nuclear magnetic resonance[24].

Intramolecular hydrogen bonding in dilute solutions of hydroxyl compounds in non-aqueous solvents can be readily detected by a downward shift in the O-H stretching frequency, the size of the shift being an approximate measure of the strength of the hydrogen bond. There is considerable evidence that intramolecular hydrogen bonding may play an important part in fixing the conformation of a molecule in non-aqueous and possibly even in aqueous solutions. The infrared spectrum of cis cyclohexane-1,3-diol in dilute solution in carbon tetrachloride shows very strong hydrogen bonding, this can only be due to the existence of an appreciable proportion of the 1,3-diaxial conformation, which is unfavoured on purely steric grounds[25]. Foster and his co-workers[26] have shown that the infrared spectrum in carbon tetrachloride of cis 5-hydroxy-2-phenyl-1,3-dioxan shows absorption at 3593 cm^{-1} for bonded hydroxyl groups only, and therefore exists exclusively in that conformation which places the hydroxyl group in an axial position (Figure 5). The trans isomer absorbs at 3633 and 3593 cm^{-1} indicative of free and bonded hydroxyl groups respectively and must therefore exist partly in the very unfavourable conformation which places the phenyl and hydroxyl groups in axial positions (Figure 6).[27]

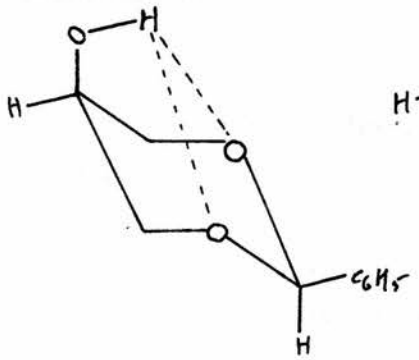


Figure 5.

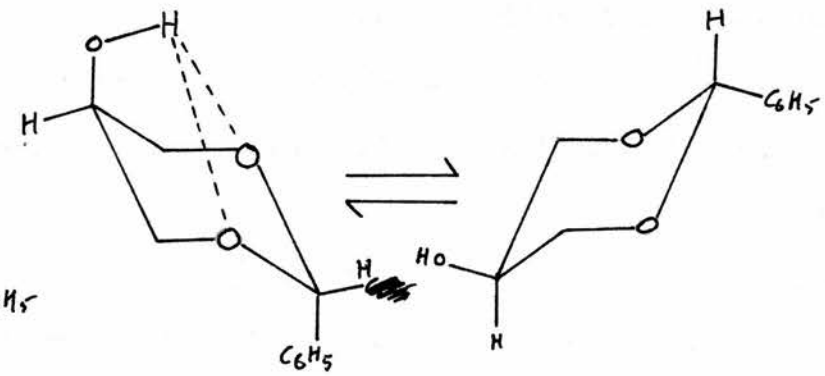


Figure 6.

The conformations of several methyl 4,6-O-benzylidene D-aldohexosides have also been examined by infrared measurements[28]. An interesting feature of such bicyclic systems is that for those compounds in which ring fusion is trans (e.g. glucosides and mannosides) the 1C conformation is sterically impossible; cis-fused compounds (eg. galactosides and idosides) are not restricted in this way.

The conformational assignments based on the various methods described above are further confirmed by the considerable success of arguments based on these conformations in the interpretation of reaction rates and equilibria in carbohydrate chemistry. Reactions which have been discussed in these terms include the oxidation of vicinal glycols with periodate[29], the formation of three-membered oxide rings from sulphonyl esters[4] and the hydrolysis of glycosides[9b]. Equilibria which can be rationalised in conformational terms include the acid-catalysed formation of 1,6-anhydro-hexoses from the parent sugars[9b] and equilibria between anomeric sugar derivatives (eg. mutarotation). One feature of the latter is of special interest in connection with the present work. Although, as pointed out by Reeves[14] the mutarotation equilibria of glucose and galactose in aqueous solution favour the equatorial β-anomer (as expected on steric grounds) the same is not true for 1-substituted derivatives. For example, equilibration of the methyl D-glucopyranosides, the penta-O-acetyl-D-glucopyranoses, or the acetohalogeno-D-glucopyranoses gives mixtures in which the axial α-anomer predominates. Edward[30] has suggested that this is due to dipole repulsions between an equatorial substituent at $C_{(1)}$ and the electron pair on the ring oxygen; his suggestion is discussed in detail later in this thesis.

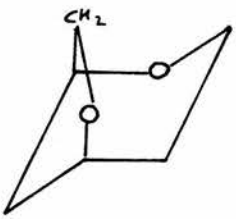
The quantitative aspect of conformation

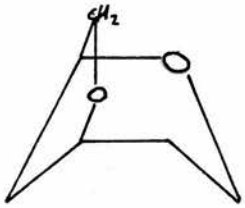
Although there is abundant evidence of the importance of conformation in explaining the transformations which sugar molecules may undergo, the quantitative assessment of the various factors involved, for example, non-bonded interactions, hydrogen bonding, dipole interactions etc., has proved difficult. In the cyclohexane series Eliel[31] and Winstein and Holness[32] and numerous other workers have demonstrated that a study of conformational equilibrium is of particular value in this respect. The rates of reaction of isomeric compounds which are known to exist in a particular conformation, as for example in the cis and trans isomers of 4-substituted tertiary butyl cyclohexanes, have been used to determine the conformational equilibrium constant for a number of derivatives and conformational free energy differences between equatorial and axial substituents have been calculated.

Nuclear magnetic resonance spectroscopy[33] and infrared spectra[34] have also been applied to the determination of conformational equilibrium.

In the inositol series Angyal and McHugh[35] have studied the conformational equilibrium between the borate ion and cis-1,3,5 triols. Free energy changes of complex formations were calculated and, for a series of compounds, the magnitudes of individual interactions were derived. Using accepted values for bond angles and bond lengths, Barker and Shaw[36] have calculated the non-bonded interactions in a series of pyranose sugars by summing the "overlaps" of the Van der Waals radii of the atoms. Although some correlation exists between the results obtained in this way and the work of Reeves, this approach suggests the fact that interatomic repulsions are not a linear function of the overlap, but increase steeply as the interatomic distance decreases.

In the present work the rate of reaction with sodium hydroxide ofthe 6-tosyl esters of a number of glycopyranosides and related compounds, the conformations of which could be predicted with some certainty, has been determined. The number of conformations of the resulting 3,6-anhydro compounds is limited to those shown below (skew forms being excluded by the presence of 3,6-anhydro-bridge).





These are equivalent to the 1C and 1B of Reeves' nomenclature. Since the 6-tosyl esters themselves are thought to exist in the C1 conformation, a conformational change must occur during the reaction and differences in rates of reaction due to conformational features would be expected. The aim of the work was to use these rate differences in an attempt to assess the relative magnitudes of some of the interactions involved.

The reaction bears a formal analogy to that between ethylene chlorohydrin and sodium hydroxide which is known to be first-order with respect to each of the reactants at low concentrations.[37] In the present case, attempts to determine second-order rate constants directly were unsuccessful, owing to kinetic complications discussed later. However, using excess sodium hydroxide pseudo first-order rate constants were determined for each compound and, from these, reasonably accurate values of the second-order rate constants were derived The differences in these rates are discussed in a qualitative manner from a

conformational point of view.

There is a natural division in the presentation of the work.

Part I describes the preparation of the 6-tosyl esters of the methyl α and β-D-glucosides and galactosides, and some related compounds.

Part II describes the kinetic work and some relevant features.

PART I

The synthesis of the 6-O-tosyl derivatives of the methyl α and β -D-glycosides of glucose, galactose and some related compounds

Discussion

Because of their great versatility in synthetic work in the field of carbohydrate chemistry much is known about the preparation and properties of the sulphonyl esters of sugars. A comprehensive review of the reactions and methods of synthesis of sulphonic esters of carbohydrates has been given by Tipson[1]. Of the various ways in which sulphonyl esters may be prepared, only one has so far achieved importance in sugar chemistry; namely the action of sulphonyl halides on suitable hydroxy compounds in the presence of an acid acceptor eg. pyridine. When mono-sulphonyl esters are required it is often necessary to protect the hydroxyl groups which are to remain unsubstituted. However, the preferential reaction of the primary hydroxyl group with acid halides is well established and in the present series of preparations of 6-tosyl esters, this method has been used almost exclusively.

Methyl 6-O-tosyl-α-D-glucoside

The earliest reported preparation of methyl 6-O-tosyl-α-D-glucoside was that of Helferich[38]. The preparation involved tritylation of the primary hydroxyl group, benzoylation of the remaining hydroxyl groups, removal of the trityl residue and finally reaction with tosyl chloride followed by removal of the benzoyl groups. Compton[39] demonstrated the preferential tosylation of the hydroxymethyl group by reacting methyl α-D-glucoside with tosyl chloride in pyridine; the reaction mixture was acetylated in situ and

methyl 2,3,4-tri-O-acetyl-6-O-tosyl-α-D-glucoside obtained as a syrup. Reaction of this material with sodium iodide in acetone solution gave crystalline methyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-glucoside. The replacement of a sulphonyloxy group by iodine in this way is of special interest in that it confirms that the group occupies the 6 position. Oldham and Rutherford[40] have shown that in the aldopyranose series of sugars only primary sulphonyl esters are affected by sodium iodide in boiling acetone, the product of the reaction in each case being the 6-deoxy-6-iodo compound. Secondary sulphonyloxy groups, in general, require more severe conditions, if they react at all. The scope and limitations of this reaction as a diagnostic tool have been discussed by Tipson.[17] Asselineau,[41] using a chromatographic technique, isolated crystalline methyl 6-O-tosyl-α-D-glucoside from the crude mixture obtained by direct tosylation of methyl α-D-glucoside in pyridine; since rather more than twice the theoretical amount of tosyl chloride necessary for monotosylation was used, the major product of the reaction was methyl 2,6-di-O-tosylα-D-glucoside. As a result of present work, methyl 6-O-tosyl-α-D-glucoside has been obtained as a crystalline dihydrate by tosylation of methyl α-D-glucoside in pyridine using a slight excess of tosyl chloride over that required for monotosylation. Isolation was achieved by means of a simplified version of a counter current extraction technique using a chloroform - water system. Subsequent to this work, the same compound has been prepared[42] by crystallisation of the crude syrup, obtained by direct tosylation of methyl α-D-glucoside, from benzene followed by a crystallisation from water.

Oxidation with sodium metaperiodate showed that two molecules of oxidising agent were consumed for each molecule of tosyl ester present, confirming that the product is methyl 6-O-tosyl-α-D-glucoside. Reaction of the methyl 6-O-

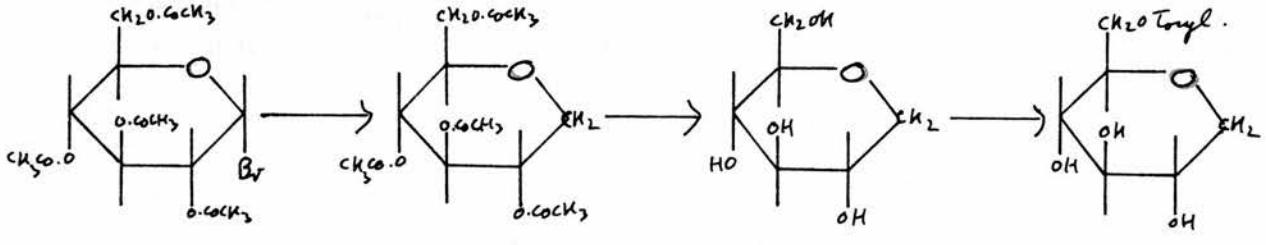
tosyl-α-D-glucoside with sodium hydroxide in alcoholic solution gave methyl 3,6-anhydro-α-D-glucopyranoside identical with that described by Haworth.[5]

Methyl 6-O-tosyl-β-D-glucoside

Methyl 6-O-tosyl-β-D-glucoside has hitherto been prepared only as a syrup[5,43]. However, crystalline methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucoside has been described by Compton.[39] In the present work, direct tosylation of methyl β-D-glucoside and isolation, using the counter current extraction procedure developed for the α anomer gave methyl 6-O-tosyl-β-D-glucoside as a crystalline monohydrate. Methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucoside prepared by the method of Compton[39] was deacetylated by the Zemplen[44] technique and after crystallisation from water gave methyl 6-O-tosyl-β-D-glucoside, identical with that obtained by the direct method. On treatment with alcoholic sodium hydroxide solution, methyl 3,6-anhydro-β-D-glucopyranoside was obtained identical with that described by Haworth.[5]

1,5-Anhydro-6-O-tosyl-D-glucitol

1,5-Anhydro-6-O-tosyl-D-glucitol was obtained by the following series of reactions:-

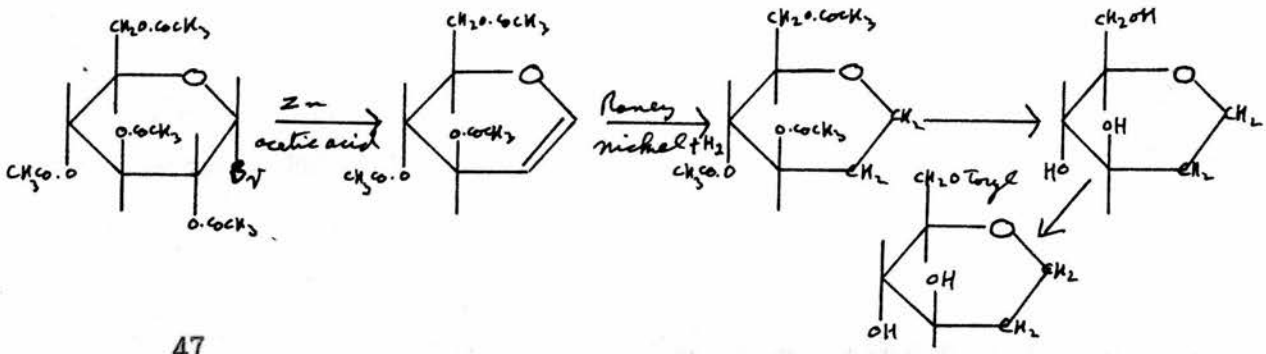


Acetobromoglucose was reduced with Raney nickel and hydrogen in presence of triethylamine. Previous workers[46] have used a platinum catalyst for this reaction, but since excessive amounts were necessary a more economical alternative seemed desirable.

Attempts to deacetylate the reduction product using the Zemplen[44] method were unsuccessful and the original alcoholic potash method of Zervas[45] was adhered to. Monotosylation of 1,5-anhydro-D-glucitol presented no difficulties and a crystalline product was readily obtained. Acetylation yielded a compound identical in optical rotation and melting point with the 1,5-anhydro-2,3,4-tri-O-acetyl-6-O-tosyl-D-glucitol described by Baker[46]. On oxidation with sodium metaperiodate under standard conditions two molecules of oxidising agent per molecule of tosyl ester were consumed as would only be expected for the 6-O-tosyl compound. Reaction of 1,5-Anhydro-6-O-tosyl-D-glucitol with alcoholic sodium hydroxide solution yielded the corresponding 3,6-Anhydro compound[46].

1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol

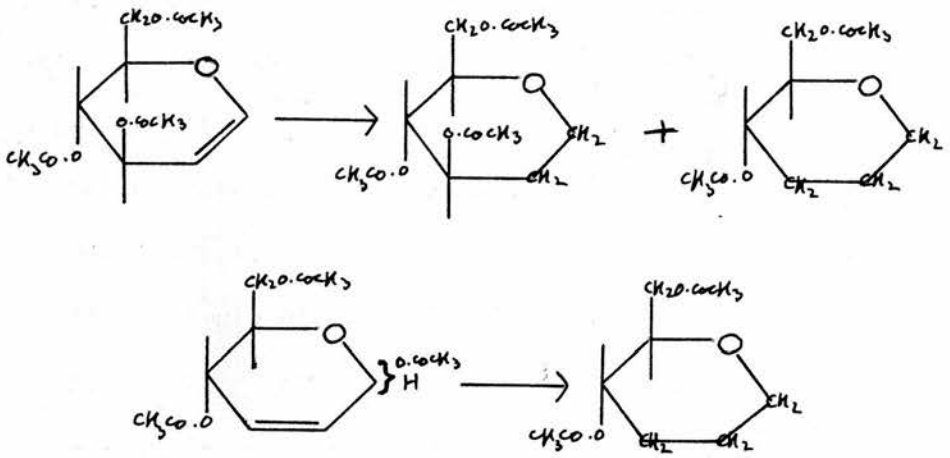
1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol was synthesised by the following series of reactions:-



Fischer[47] obtained 3,4,6-tri-O-acetyl-1,5-anhydro-2-deoxy-D-glucitol in excellent yield by catalytic reduction of 3,4,6-tri-O-acetyl-D-glucal in acetic acid using platinum black as catalyst. Hydrolysis of the product gave 1,5-anhydro-2-deoxy-D-glucitol in good yield. More recently Foster, Stacey and Vardheim[48] have obtained the same product using platinum oxide in methanol as catalyst.

In the present work tri-O-acetyl-D-glucal obtained in excellent yield

by Fischer's original method was reduced at atmospheric temperature and pressure using Raney-nickel in methanol as catalyst and the tri-O-acetyl compound deacetylated by the Zemplen technique. Attempts to carry out the reduction using platinum oxide catalyst in either methanol or acetic acid resulted in an uptake of more than the two atoms of hydrogen per molecule required for the formation of tri-O-acetyl-1,5-anhydro-D-glucitol; in methanolic solution twice this amount was consumed. This result suggests that under these conditions hydrogenolysis of the 3-acetoxy group occurred.

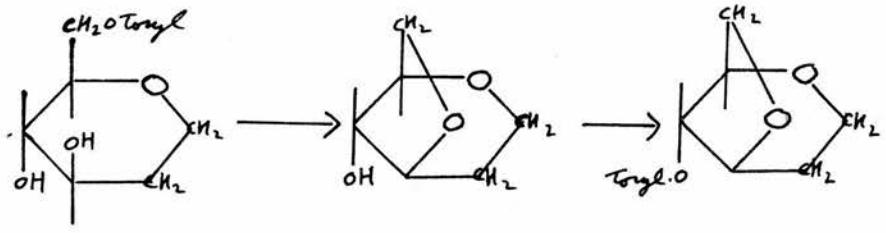


The removal of acetoxy groups in this way by hydrogenolysis of allylic esters is well known. Although no crystalline products could be obtained, the syrupy material produced on hydrolysis gave a small amount of a benzylidene derivative, identical in melting point with that obtained by Bergmann and Breuers[49] from 1,5-anhydro-2,3-dideoxy-D-glucitol which is the main product of the reduction of tri-O-acetyl-pseudo-D-glucal with palladium black in acetic acid.

The preparation of 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol was achieved by direct tosylation of 1,5-anhydro-2-deoxy-D-glucitol giving a crystalline monotosyl compound in good yield. The optical rotation $[\alpha]_D^{20}$ + 8.4° (c1.8 in $CHCl_3$); m.p. 110-111.5° of this compound was in good agreement with that

observed by Foster[48] $[\alpha]_D$ + 8.5° (c 1.0 in $CHCl_3$); m.p. 104°.

1,5:3,6-Dianhydro-2-deoxy-D-glucitol was obtained as a deliquescent, crystalline solid by the action of alcoholic sodium hydroxide on the 6-O-tosyl compound. The 3,6-Anhydro derivative was not oxidised by sodium metaperiodate under standard conditions and yielded a highly crystalline compound analysing correctly for 1,5:3,6-Dianhydro-2-deoxy-4-O-tosyl-D-glucitol when treated with tosyl chloride in pyridine.



Periodate oxidation of 1,5-anhydro-2-deoxy-6-O tosyl-D-glucitol and the parent 1,5-anhydro-2-deoxy-D-glucitol gave anomalous results when the periodate was determined by the sodium arsenite - potassium iodide method of Müller and Friedberger[50]. Under the mildly alkaline conditions, the liberated iodine is consumed rapidly and the solution becomes colourless. Similar results have been reported for oxidations in which malondiadehyde derivatives are formed[51] and more recently for the oxidation of some 2-deoxy-sugars[52].

Titration of the oxidation products with standard iodine solution in presence of sodium bicarbonate showed that approximately 5.5 equivalents of iodine were consumed for each molecule of tosyl ester present in the original solution. The final solution deposited a small amount of yellow solid on standing and the characteristic smell of iodoform was observed although the presence of this substance was not confirmed.

The oxidation products did not react with iodine under acid conditions. Acidification of the solution after oxidation, followed by the addition of potassium iodide and titration of the liberated iodine with sodium thiosulphate, proved to be a satisfactory method of periodate analysis. One molecule of sodium metaperiodate was consumed for each molecule of sugar ester present as would be expected for 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol.

Methyl 6-O-tosyl-α-D-galactoside

1,2:3,4-di-isopropylidene-D-galactose is easily prepared and provides a ready method for the preparation of 6-O-tosyl-1,2:3,4-di-isopropylidene-D-galactose. Ohle and Thiele[53] prepared methyl 6-O-tosyl-α-D-galactoside by the action of methanolic hydrochloric acid on 6-O-tosyl-β-D-galactose obtained by deacetonation of the di-isopropylidene compound. Later Bell and Williamson[54] simplified this method by using the direct action of methanolic hydrogen chloride on the di-isopropylidene-6-O-tosyl compound. Elderfield[55], Haworth[6], and numerous other workers[56] have prepared methyl 6-O-tosyl-α-D-galactoside directly by the monotosylation of methyl α-D-galactoside in pyridine.

There is considerable variation in the decomposition melting points given by different workers for the tosylate. In general, direct tosylation of methyl α-D-galactoside produces higher melting products; in this way Haworth[6] prepared material m.p. 188° (decomp), $[\alpha]_D^{17}$ + 118° (c1.400 in pyridine) and other workers using this route obtained m.p. 175° [56a], 180° [56b] and 172° [55] respectively. The method of Bell & Williamson on the other hand produced material m.p. 170° [54] $[\alpha]_D^{20}$ + 103.5° (pyridine).

In the present series of experiments, methyl 6-O-tosyl-α-D-galactoside was prepared by both routes and in agreement with the foregoing, a somewhat

higher melting product (173-174°) was obtained by direct tosylation than via the di-isopropylidene compound (165°), even when the 6-O-tosyl compound obtained from the latter was purified via its crystalline 2:3:4-tri-O-acetyl derivative. However, the optical rotation of material obtained from either source was $[\alpha]_D$ + 111.5° (c1.402 in pyridine). Further, it was found that, if low melting material was crystallised from ~~aqueous~~-pyridine/LIGHT PETROLEUM, a high melting product was obtained, and that conversely, if the substance obtained by direct tosylation was crystallised from solvents other than pyridine, there was a tendency for low melting products to occur. It is suggested that traces of pyridine may be responsible for the higher melting point, the function of the pyridine being to inhibit the decomposition which may otherwise be accelerated by traces of acid formed.

Methyl 6-O-tosyl-β-D-galactoside,

Haworth[6] obtained methyl 6-O-tosyl-β-D-galactoside, m.p. 137°, $[\alpha]_D$ -3.5° (c0.8 in pyridine) from 6-O-tosyl-D-galactose by a four-stage synthesis via the acetobromo compound.

In the present work methyl 6-O-tosyl-β-D-galactoside was prepared by direct tosylation of methyl β-D-galactoside in dry pyridine. Some variability was observed in the melting point of the products and there is some evidence that this may be due to the formation of an ill-defined hydrate under certain conditions of isolation. Benzoylation of the product gave a crystalline material analysing correctly for methyl 2,3,4-tri-O-benzoyl-6-O-tosyl-β-D-galactoside,

EXPERIMENTAL

Methyl 6-O-tosyl-α-D-glucoside.

Methyl α-D-glucoside (9.7 g.), dried in vacuo over phosphorous pentoxide at 100°, was dissolved in dry pyridine (75 ml.) and cooled in an ice-salt mixture. A solution of tosyl chloride (10.5 g.) in dry pyridine (50 ml.) was then added over 1½ hrs., keeping the temperature below 5°. The mixture was kept at 0-5° for 3 hrs. and then allowed to stand at room temperature overnight. Excess tosyl chloride was decomposed by cooling the mixture to 0° and adding water (25 ml.) slowly. After 1 hr. the mixture was poured into a solution of sodium bicarbonate (4.63 g.) in cold water (500 ml.) and the resulting solution evaporated to dryness in vacuo; the last traces of water were removed by azeotropic distillation with benzene (100 ml.).

The gummy residue was dissolved in chloroform (100 ml.) and the mixture filtered to remove insoluble inorganic material. The chloroform solution (A) was shaken with ice-cold water (100 ml.) and the aqueous phase (I) allowed to separate; separation was rather slow and the mixture required to be left overnight. The chloroform layer (A) was then separated off and the aqueous phase (I) shaken with a similar quantity of chloroform (B). (I) was again separated and shaken with chloroform (100 ml.) (C). Evaporation to dryness of (I) after a final separation yielded only traces of inorganic and other readily water-soluble material. A second quantity of water (100 ml.) (II) at 25° was then shaken successively with (A), (B) & (C) and the final solution evaporated to dryness in vacuo at 40°. Water (2 ml.) was added to the syrupy residue which crystallised readily on scratching. The extraction of the chloroform solution with successive quantities of water was continued until

the final aqueous extract contained a negligible quantity of solid on evaporation: the total volume of water used in the extraction was 1500 ml. The aqueous extracts were evaporated separately to small bulk and allowed to crystallise. After filtration, the fractions were combined and allowed to dry in air (yield 8.14 g, m.p. 53-54°). The crude product was twice crystallised from water (25 ml.) and allowed to dry in air (yield 6.7 g, m.p. 55-56°). The air dried product (4.876 g.) was heated in vacuo over phosphorous pentoxide at 40° giving anhydrous methyl 6-O-tosyl-α-D-glucoside (4.426 g.), $[\alpha]_D^{18.5}$ + 98.4° (c 0.665 in H_2O), lit $[\alpha]_D^{20}$ + 98.5 (EtOH) [42] (Found: C,48.6: H,5.7: S,9.3. Calc. for $C_{14}H_{20}O_8S$: C,48.3; H,5.8; S,9.3%).

The loss in weight corresponds to 1.97 molecules of water of crystallisation.

Periodate oxidation was carried out by the method of Müller and Friedberger [50] using 0.025M sodium metaperiodate solution: uptake 1.99, 2.00 mol (24 hr.).

Methyl 3,6-anhydro-α-D-glucopyranoside.

Methyl 6-O-tosyl-α-D-glucoside (5 g.) was dissolved in absolute alcohol (32 ml.) and sodium hydroxide solution (16 ml. 1.0N) added. The product was isolated using Haworth's conditions[5]. The crystalline residue (2.22 g, m.p. 94-102°) was dissolved by warming with benzene (10 ml.) and the solution filtered to remove insoluble material. On cooling, crystalline material (1.55 g, m.p. 99-103°) was obtained. The material was twice crystallised from ethyl acetate giving methyl 3,6-Anhydro-α-D-glucopyranoside, m.p. 106.5 -109°).

Methyl 6-O-tosyl-β-D-glucoside.

Methyl β-D-glucoside hemihydrate (20.3 g.), dried in vacuo at 100° over phosphorous pentoxide, was dissolved in dry pyridine (150 ml.) and the mixture

cooled to 0°. A solution of tosyl chloride (21 g.) in dry pyridine (100 ml.) was then added over 1 hr. and the temperature kept below 0° for 3 hrs. After standing overnight, the mixture was recooled to 0° and water (50 ml.) added slowly to hydrolyse the excess tosyl chloride. The mixture was poured into water (1000 ml.) containing sodium bicarbonate (9.3 g.) and the resulting solution evaporated to a viscous syrup in vacuo. The syrup was dissolved in chloroform (200 ml.) and after filtration was subject to the extraction process described for the alpha - anomer. On evaporation of the aqueous extracts to small bulk in vacuo, crystalline solid separated which was removed by filtration and allowed to dry in air.

Frn. I, inorganic material and sodium tosylate; Frn.II and Frn. III, 6.15 g, of crystalline material, m.p. 69.5-70.5°; Frn. IV, 5.82 g, m.p. 70-71°; Frn. V, 2.95 g, m.p. 69.5-71°; Frns. VI, VII and VIII, 3.11 g, m.p. 69.5-71°; Frn. IX, trace of solid. The fractions were combined, recrystallised from water and allowed to dry in air $[\alpha]_D^{18}$ -15.1° (c 0.797 in H_2O). (Found: C,45.6; H,5.7; S,8.4. $C_{14}H_{20}O_8S.H_2O$ requires C,45.9; H,6.0; S,8.73%).

The air dried material (1.303 g.), dried in vacuo at 40-45° over phosphorous pentoxide, gave a product analysing correctly for anhydrous methyl 6-O-tosyl-β-D-glucoside (Found: C,49.4; H,5.8; S,9.0. $C_{14}H_{20}O_8S$ requires C,48.3; H,5.8; S,9.2%). The loss in weight on drying (0.0648 g.) corresponds to one molecule of water of crystallisation.

Methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucoside.

Methyl β-D-glucoside hemihydrate (5 g.) was dried by heating in vacuo over phosphorous pentoxide at 77° for 3 hrs. The dried material was dissolved in dry pyridine (50 ml.) and cooled to -10° in an ice-salt mixture. Tosyl chloride (5.39 g.) in dry pyridine (50 ml.) was cooled to 0° and added rapidly

to the solution of the glucoside; the mixture was shaken vigorously to ensure good mixing. After keeping below 0° for 3 hrs. in an ice-salt bath, the solution was allowed to come to room temperature gradually and left overnight. It was then recooled to 0° and acetic anhydride (40 ml.) added. The solution was maintained at 0° for 2 hrs. and again left at room temperature overnight. Finally the mixture was cooled to 0°and water (10 ml.) added dropwise. Chloroform (50 ml.) was then added and the mixture poured into cold water (200 ml.). The chloroform layer was separated and the aqueous phase washed with two further amounts of chloroform (20 ml.). The combined chloroform extract was washed with iced water, 10% sulphuric acid, saturated sodium bicarbonate solution and finally cold water. After drying over sodium sulphate, the chloroform was removed in vacuo and the residual syrup crystallised from absolute alcohol (20 ml.). The crude triacetate was filtered off and after successive recrystallisation from absolute alcohol and benzene-light petroleum, the product (2.1g.) had m.p. 166.5-167.5°, $[\alpha]_D^{22}$ + 7.1° (c4.104 in $CHCl_3$) lit: m.p. 169-170°, $[\alpha]_D^{30}$ + 7.4° (c3.654 in $CHCl_3$).[39] Methyl tetra-O-acetyl-β-D-glucoside (2.7 g.) m.p. 101-103°, mixed m.p. 100-102 (lit. m.p. 104-105°)[57] was obtained by evaporation of the filtrates and crystallisation of the residue from benzene-light petroleum.

Deacetylation of methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucoside

Methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucoside (2,3 g.) was shaken for 48 hrs. with absolute methanol (80 ml.) containing sodium methoxide solution (8ml., 0.05M). The faint residual alkalinity was removed by passing the solution through a column of Amberlite IRC-50(H) resin and the eluate was evaporated to dryness at 35-40° under reduced pressure. The residue crystallised on standing overnight with a small quantity of water and was

isolated by filtration. After several crystallisations from water methyl 6-O-tosyl-β-D-glucoside (1.17 g, m.p. 69-70°) was obtained.

Methyl 3,6-anhydro-β-D-glucopyranoside

Methyl 6-O-tosyl-β-D-glucoside (5 g,) was treated with alcoholic sodium hydroxide according to the method of Haworth[5]. The crude syrup on vaccum distillation gave methyl 3,6-anhydro-β-D-glucopyranoside (1,6 g,) as a colourless extremely hygroscopic syrup (b.p. 130-140°/011 mm.) which crystallised on standing in a vacuum desiccator.

2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol

Acetobromoglucose (28.65 g,) was dissolved in ethyl acetate (200 ml.) to which triethylamine (7 g,) and a slurry of Raney-nickel (15 g,) in ethyl acetate was then added. The mixture was hydrogenated at atmospheric pressure and room temperature for 18 hrs. after which the uptake of hydrogen ceased. It was then filtered to remove catalyst. After washing the filtrate twice with cold water (100 ml.), the ethyl acetate was evaporated under reduced pressure at 40°. The residue was dissolved in ether (50 ml.) and on further evaporation to dryness gave a residue (20 g,) which solidified on scratching (m.p.56-62°). Repeated crystallisation of a sample from aqueous methanol gave material with m.p. 64-66° cf. Richtmyer and Hudson[58], m.p. 74° .

1,5-Anhydro-D-glucitol

Crude 2,3,4,6-tetra-O-acetyl-1,5-anhydro-D-glucitol-(20 g,) was dissolved in absolute alcohol (100 ml.), a solution of potassium hydroxide (17.5 g,) in absolute alcohol (150 ml.) was added and the mixture allowed to stand at room temperature for 48 hrs. Sulphuric acid (135 ml. 2.34N) was then added and, after filtration to remove the precipitated potassium sulphate, the solution

was evaporated in vacuo at 40° to a viscous syrup. The syrup was dissolved in methanol (100 ml.) and the acid solution neutralised by addition of methanolic potassium hydroxide solution. Charcoal was added to the mixture which was then filtered and the filtrate evaporated under reduced pressure. The solid residue was dissolved in methanol (100 ml.) and filtered through Celite; after concentration the product crystallised on standing. The material was filtered off and twice crystallised from methanol to yield 1,5-anhydro-D-glucitol (4.6 g.), m.p. 139-140 (lit:. 140-141° [59]; 142° [60]. A further quantity (1.47 g.) of material m.p. 137-139° was obtained by concentrating the mother liquor from the final crystallisation in vacuo to half its volume.

1,5-Anhydro-6-O-tosyl-D-glucitol

1,5-Anhydro-D-glucitol (5.9 g.) was dried in vacuo overnight and dissolved by stirring with dry pyridine (54 ml.). The solution was cooled in an ice-salt mixture and tosyl chloride (7.55 g.) in pyridine (36 ml.) was added dropwise over 1 hour with mechanical stirring. The solution was kept at 0° for 3 hours and then left at room temperature overnight. The excess tosyl chloride was decomposed by cooling the mixture to 0° and adding water (18 ml.) cautiously. After 1 hour the solution was poured into water (360 ml.) containing sodium bicarbonate (3.33 g.) and the mixture was evaporated to dryness at 40° under reduced pressure. After shaking the solid residue with chloroform (100 ml.) and filtering, the residue was dissolved in boiling water (80 ml.), a little carbon was added and the hot solution screened. The product crystallised on standing overnight and was filtered off, washed with a little cold water and dried in a vaccum desiccator giving 1,5-anhydro-6-O-tosyl-D-glucitol (4.35 g, m.p. 167-168°).

The remaining chloroform solution was extracted several times with 100 ml. quantities of warm water. On evaporation to dryness the aqueous extracts yielded 1.1 g, of material which after further crystallisation from water had m.p. 167-168° (0.99 g,).

The main product was crystallised several times from water giving a substance m.p. 167.5-168.5°, $[\alpha]_D$ + 43.5° (c 1.01 in pyridine) (Found: C,49.0; H, 5.8; S,10.3 $C_{13}H_{18}O_7S$ requires C,49.1; H,5.7; S,10.1%). Periodate oxidation was carried out by the standard method[50] using 0.025M sodium metaperiodate. Uptake: 2.014, 2.068 moles (24 hr.).

2,3,4-Tri-O-acetyl-1,5-anhydro-6-O-tosyl-D-glucitol

1,5-Anhydro-6-O-tosyl-D-glucitol (100 mg.) was dissolved in pyridine (5 ml.) and the solution cooled to 0°. Acetic anhydride (1 ml.) was added and the solution allowed to stand overnight. The solution was poured into cold water (50 ml.) and the insoluble product extracted with chloroform. After washing with dilute hydrochloric acid and water, the solution was evaporated to dryness and the residue extracted with light petroleum. The insoluble material remaining was crystallised from aqueous alchol giving the triacetate (115 mg.) m.p. 143°, $[\alpha]_D^{20}$ + 58.7° (c 0.818 in $CHCl_3$)[46].

3,4,6-Tri-O-acetyl-D-glucal

This was prepared by the method of Fischer and Zach[47] giving a crude product (72%) m.p. 52-53°. After crystallisation from absolute alcohol-light petroleum, material with m.p. 54-54.5° (lit.: 54-55°) was obtained.

1,5-Anhydro-2-deoxy-D-glucitol

3,4,6-Tri-O-acetyl-D-glucal (25 g,) was dissolved in methanol (80 ml.) and a slurry of Raney-nickel (9 g,) in methanol added. The material was

hydrogenated at room temperature (14.5°) and atmospheric pressure (753.5 mm. Hg.) for 5½ hours until the uptake of hydrogen (2300 ml.) ceased. After filtration to remove the catalyst, the solution was evaporated to dryness at 40° under reduced pressure. The syrupy tri-O-acetyl-1,5-anhydro-2-deoxy-D-glucitol was dissolved in dry methanol (250 ml.) and sodium methoxide solution (2.5 ml., 1.0N) added. After standing overnight at room temperature, traces of alkali were removed from the solution by passing it through a column of Amberlite IRC-50 resin. The resulting solution was evaporated to a syrup in vacuo and gave large crystalline plates (12.9 g, 94.5%) on seeding and standing overnight in a desiccator. After two crystallisations from absolute alcohol-light petroleum, 1,5-anhydro-2-deoxy-D-glucitol (5.7 g, m.p. 83.5 - 84.5°; lit. m.p. 86-87°) was obtained. The very hygroscopic crystals were kept in a desiccator.

1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol

1,5-Anhydro-2-deoxy-D-glucitol (5.5 g,) was dried in vacuo at 40° over phosphorous pentoxide for 18 hrs. and then dissolved in dry pyridine (56 ml.). The solution was cooled in an ice-salt mixture and tosyl chloride (7.8 g,) in dry pyridine (36 ml.) added to the well stirred mixture over 1 hr., care being taken to exclude moisture. The mixture was maintained at 0° for 5 hrs. and then at room temperature for 18 hrs. The excess tosyl chloride was decomposed by recooling the mixture to 0° and adding cold water (20 ml.). The solution was distilled to dryness in vacuo at 40° and the residue dissolved in chloroform (125 ml.). Insoluble inorganic material was removed by filtration and the chloroform solution extracted three times with cold water (100 ml.). The combined aqueous extracts were washed with chloroform (3x100 ml.) and after drying over sodium sulphate the combined chloroform solutions were evaporated to dryness

in vacuo leaving a crystalline residue. Crystallisation from benzene (20 ml.) gave 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol (6.65 g, m.p. 107-109°) which after successive recrystallisations from benzene and chloroform-light petroleum, gave material m.p. 110.5-111.5°, $[\alpha]_D^{20}$ + 8.4° (c1.8 in $CHCl_3$) (Found: C,51.7; H,6.0; S,10.5, calculated for $C_{13}H_{18}O_6S$: C,51.7; H,6.0; S,10.6%).

Periodate uptakes were determined using acidified potassium iodide and sodium thiosulphate.[61] Uptake of 0.025N sodium metaperiodate; 1,108, 1,089 moles (40 hr.).

1,5:3,6-Dianhydro-2-deoxy-D-glucitol

1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol (1 g,) was dissolved in absolute alcohol (6 ml.) and sodium hydroxide solution (3 ml., 1.0N) added. After standing overnight at room temperature, the faintly alkaline mixture was treated with carbon dioxide and the solution evaporated to dryness under reduced pressure. The residue was extracted several times with hot acetone and the combined extracts re-evaporated. The syrupy residue was dissolved in hot ether (50 ml.) and screened from insoluble material. On dilution with an equal volume of light petroleum (60-80°), the solution deposited feathery crystals (70 mg.) m.p. 110-111°, undepressed by 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol. The filtrates were evaporated to dryness and the residue distilled in vacuo giving the dianhydro-compound as a colourless syrup (270 mg. b.p. 110-120°/0.01 mm.), the bulk of which crystallised on standing overnight in a desiccator. The optical rotation of the syrupy, very hygroscopic crystals, after drying on filter-paper in a desiccator over phosphorous pentoxide, was $[\alpha]_D^{20}$ - 27.2° (c 2.207 in H_2O). Periodate oxidation using standard conditions[50] showed a negligible uptake.

1,5:3,6-Dianhydro-2-deoxy-4-O-tosyl-D-glucitol

1,5:3,6-Dianhydro-2-deoxy-D-glucitol (86 mg.) was dissolved in pyridine

(2 ml.) and cooled in an ice-salt mixture. Tosyl chloride (250 mg.) was added and the mixture was kept at 0° for 3 hrs. and then at room temperature overnight. Excess tosyl chloride was destroyed by recooling to 0° and adding cold water (0.5 ml.). On pouring the mixture into cold water (10 ml.), a crystalline solid separated which was extracted with chloroform (3 x 10 ml.). After washing the chloroform extract successively with ice-cold 10% sulphuric acid solution, saturated sodium bicarbonate solution and finally water, the chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. The solid residue (170 mg.) was recrystallised three times from absolute alcohol giving 1,5:3,6-dianhydro-2-deoxy-4-O-tosyl-D-glucitol (85.3 mg.), m.p. 151-151.5°, $[\alpha]_D$ - 26.6° (c 0.3768 in pyridine) (Found: C,55.5; H,5.77; S,11.5. $C_{13}H_{16}O_5S$ requires: C,54.9; H,5.6; S,11.3%).

Methyl 6-O-tosyl-α-D-galactoside

1) Preparation from di-isopropylidene-D-galactose-6-tosylate

Di-isopropylidene-D-galactose-6-tosylate, prepared by the method of Bell and Williamson[54] was dissolved in methanol (180 ml.) containing 2% of dry hydrogen chloride. On standing overnight, crystalline material separated which was isolated by filtration and washed with methanol. The product (5.7 g, m.p. 158-160°, decomp.) was crystallised from aqueous alcohol giving methyl 6-O-tosyl-α-D-galactoside (3.53 g, m.p. 164° decomp.). On standing for a month the methanolic hydrogen chloride solution gradually deposited further crystals which were isolated in a similar manner giving a product (5.65 g, m.p. 164°, decomp.). The combined products were dissolved in dry pyridine (50 ml.) and cooled to 0° in an ice bath. Acetic anhydride (30 ml.) was added keeping the temperature below 5° for 3 hrs. After standing overnight the mixture was cooled to 0° and water (10 ml.) added cautiously to decompose the excess acetic anhydride. The

mixture was then poured into cold water (500 ml.) and after decantation of the liquors, the residual gum was triturated several times with cold water and finally with alcohol which gave a crystalline mass. Water was then added and the product filtered off and washed well with cold water, giving, on drying in air, a product (11.3 g.), m.p. 124°. After several crystallisations from methanol and finally absolute alcohol methyl 2,3,4-tri-O-acetyl-6-O-tosyl-α-D-galactoside (7.8 g.), m.p. 127° was obtained.

The material was deacetylated by dissolving in dry methanol (360 ml.) and adding sodium methoxide (0.5 ml., 1,0N) solution. Crystalline material separated and after 24 hrs. the product was filtered off, washed with a little methanol and allowed to dry in air giving methyl 6-O-tosyl-α-D-galactoside (4.5 g, m.p. 165°, decomp.), $[\alpha]_D$ + 111.5° (C 1.402 in pyridine). The melting point was unchanged on crystallisation from aqueous alcohol.

On addition of light petroleum to the pyridine solution used for optical measurements, the dissolved sugar was precipitated. This material was filtered off, washed with a little petroleum-ether and dried in a vacuum desiccator. The product had m.p. 174° (decomp.).

2) By direct tosylation of methyl α-D-galactoside

Methyl α-D-galactoside (19.4 g.) (dried over P_2O_5 at 80°) was dissolved in anhydrous pyridine (50 ml.) and cooled in an ice bath. A solution of tosyl chloride (21 g.) in dry pyridine (50 ml.) was added dropwise over 2 hrs. and the mixture kept at 0° for 2 - 3 hrs. and at room temperature overnight. Excess tosyl chloride was decomposed by cooling to 0° and adding water (25 ml.) cautiously The mixture was then poured into cold water (2 litres). Gummy material separated and the liquors were decanted and placed in a refrigerator overnight. A crystallim

compound separated which was filtered off, washed well with water and dried in a vacuum desiccator giving a product (8.7 g,) m.p. 169° (decomp.). The gummy residue solidified on adding more water and scratching; it was filtered off, washed with water and dried in the same way giving a product (10.8 g,) m.p. 166° (decomp.). This latter material on crystallisation from aqueous methanol gave a product m.p. 160° (decomp.) which on recrystallisation from pyridine-light petroleum had m.p. 173-174° (decomp.) $[\alpha]_D$ + 111.4° (c1,395 in pyridine).

Methyl 6-O tosyl-β-D-galactoside

Methyl β-D-galactoside (9.7 g,) (dried in vacuo over P_2O_5 at 40°) was dissolved in dry pyridine (100 ml.). To the solution at 0° was added a solution of tosyl chloride (10,5 g,) in dry pyridine (50 ml.). After keeping the mixture at 0° for 2-3 hrs., the temperature was allowed to rise and the mixture left overnight. It was then recooled to 0° and water (10 ml.) added to destroy the excess tosyl chloride; after 1 hr. the solution was poured into cold water (500 ml.) containing sodium bicarbonate (4.75 g,) and distilled to dryness under reduced pressure at 40°. The dry residue was extracted with chloroform (2 x 100 ml.) and the combined extracts subjected to an elementary counter-current procedure. The chloroform solution (A) was shaken with an equal volume of water (I) and allowed to separate. The aqueous phase was then shaken with two successive amounts of chloroform (B and C) and evaporated to dryness in vacuo giving a residue (0.87 g,) m.p. 121-124° (decomp.). A second quantity of water (II) was then shaken with (A) (B) and (C) in turn; during the course of this operation crystalline material separated out and was filtered off giving, when dried in air, material (1.24 g,) m.p. 119-122°. (II) was evaporated to small bulk in vacuo and the crystalline material which separated was filtered off giving

material (1,16 g,) m.p. indef. ca. 90°. The products from (B) and (C) were combined (0.3 g,) m.p. indef. ca. 119°. The chloroform filtrates were again extracted with three further amounts of water from which material (0.98 g,) m.p. ca. 120°, was obtained on evaporating to small bulk in vacuo. The combined material obtained from the aqueous extracts was twice crystallised from absolute alcohol giving a product (1.93 g,) m.p. 129-130°, after drying in a vacuum desiccator. The remaining fractions were combined and similarly crystallised giving material (3.62 g,) m.p. 129-130° $[\alpha]_D$ - 6.9° (c 0.9985 in pyridine) (Found: C,48.4; H,5.8; S,9.0 Calculated for $C_{14}H_{20}O_8S$; C,48.3; H,5.8; S,9.23). (lit. m.p. 137°, $[\alpha]_D$ ca. - 3.5 (0.8 py.).[6]

A sample of the substance (0.52 g,) was crystallised from water (5 ml.) giving well-defined crystals which on allowing to dry in air had m.p. 109-110°. When dried in vacuo over phosphorous pentoxide in vacuo at 40°C, these lost weight corresponding to 1.25 molecules of water of crystallisation.

Methyl 2,3,4-tri-O-benzoyl-6-O-tosyl-β-D-galactoside

Methyl 6-O-tosyl-β-D-galactoside (200 mg,) was dissolved in dry pyridine and benzoyl chloride (5 ml.) added, the temperature being maintained at 0° by means of an ice-bath for 3-4 hrs. After standing for a further 48 hrs. at room temperature, the mixture was recooled and water (2 ml.) added, the whole was then poured into cold water (100 ml.) and left standing for 1 week during which time the oily material solidified. The solid was extracted with chloroform and the extract washed with 2N sulphuric acid followed by sodium bicarbonate. Evaporation of the dried chloroform solution in vacuo gave a solid residue which crystallised readily from absolute alcohol. Further crystallisation from benzene-light petroleum and finally absolute alcohol yielded the tribenzoate (250 mg.), m.p. 192-194° (decomp.) $[\alpha]_D$ + 65.7 (c 0.8984 in pyridine) (Found: C,63.1; H,4.9; S,5.2. $C_{35}H_{32}O_{11}S$ requires: C,63.6; H,4.9; S,4.9%.)

PART II

Investigation of the kinetics of the reaction of the 6-tosyl esters of a number of pyranosides with sodium hydroxide

A. The products of the reaction

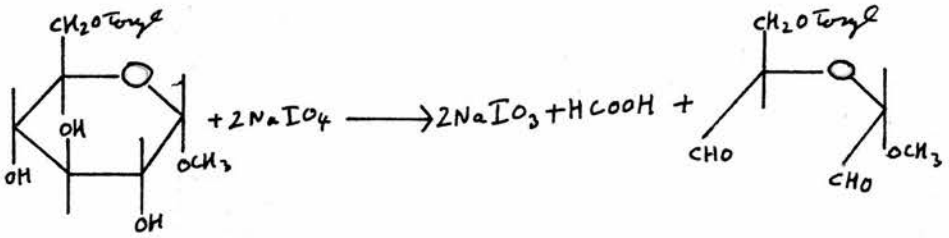
In any investigation into the reaction mechanism and kinetics of a chemical process, a complete and quantitive determination of the nature of the reaction products is essential. The formation of a 3,6-anhydro compound when the 6-tosyl ester or 6-deoxy-6-halogeno compound or nitrate ester of a sugar having suitable orientated groups is treated with sodium hydroxide is well known. Haworth and his co-workers have described the preparation of methyl 3,6-anhydro-α and β-D-glucopyranosides[5] and of methyl 3,6-anhydro-α and β-D-galactopyranosides[6] by the action of alcoholic sodium hydroxide solution on the 6-O tosyl ester of the corresponding glycoside. S.B. Baker[46] prepared 1,5:3,6-dianhydro-D-glucitol (D - neoglucide) by the action of sodium methoxide in methanolic solution on 2,3,4-tri-O-acetyl-1,5-anhydro-6-O-tosyl-D-glucitol and demonstrated the inertness of the anhydro compound towards oxidation by lead tetra-acetate. The present work has shown the formation 1,5:3,6-dianhydro-2-deoxy-D-glucitol when 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol is treated with sodium hydroxide in aqueous alcohol. However, because of the ease with which some of the products undergo intramolecular rearrangement reactions and since many of the compounds are hygroscopic and difficult to handle, the yields obtained were far from quantitive.

An alternative way of demonstrating the exclusive formation of 3,6-anhydro compounds was therefore devised.

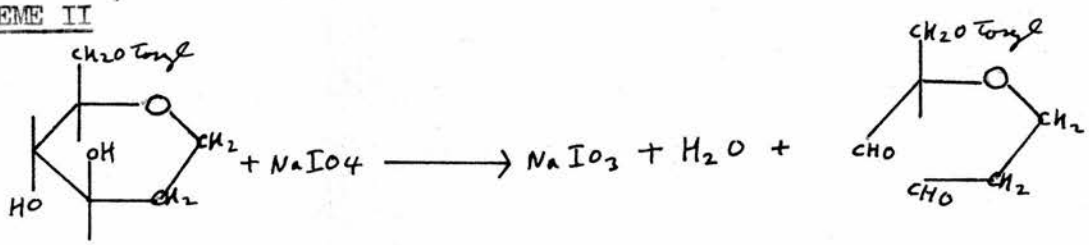
The methyl 6-O-tosyl pyranosides of glucose and galactose, and the 6-O-tosyl derivative of 1,5-anhydro-D-glucitol react smoothly with sodium periodate; the vic- (or α) glycol groupings present are cleaved and two molecules of sodium

periodate are consumed quantitatively in accordance with scheme I. Similarly 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol consumes one molecule of sodium periodate as in scheme II.

SCHEME I



SCHEME II



The corresponding 3,6-anhydro compounds contain no vic-glycol grouping and therefore would not be expected to react with sodium periodate. In order to confirm, by experiment, that 3,6-anhydro compound is formed exclusively when the corresponding 6-O-tosyl-ester is treated with sodium hydroxide, methyl 6-O-tosyl-α-D-glucoside was treated with an excess of sodium hydroxide, the pH of the solution was then adjusted to 4.45 with sulphuric acid and an acetate buffer. With sodium periodate the resulting solution showed a significant uptake of oxidant which increased regularly with increasing time of oxidation (eg. 0.037,0.047 moles (90 hr.); 0.117, 0.121 moles (230 hr.). Control experiments in which methyl 3, 6-anhydro-α-D-glucopyranoside was treated similarly also showed an appreciable uptake of sodium metaperiodate.

Consistent results were obtained when, after treating the methyl 6-O-tosyl-α-D-glucoside with sodium hydroxide, the solution was adjusted to pH 6.64 with a phosphate buffer and sulphamic acid, [redacted]. Under these conditions there

was no significant uptake of sodium metaperiodate with oxidation times of up to 168 hr. Blank experiments showed that methyl α-D-glucoside was oxidised quantitatively in 24 hr. under these conditions.

When examined by the above method, the reaction products of methyl 6-O-tosyl-α and β-D-glucoside and methyl 6-O-tosyl-α and β-D-galactoside, 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol and 1,5-anhydro-6-O-tosyl-D-glucitol with sodium hydroxide showed a negligible uptake of sodium periodate from which it was concluded that the 3,6-anhydro sugar was the sole product in each case.

EXPERIMENTAL.

The action of sodium periodate at pH 4.45 on the reaction products of methyl 6-O-tosyl-α-D-glucopyranoside with excess sodium hydroxide.

20.25 mg. samples of tosyl compound were weighed accurately and dissolved in water (5 ml.). Sodium hydroxide solution (5 ml., 0.8N) was then added to each sample and the solutions allowed to stand at room temperature for 2½ hr. They were then adjusted to pH 4.45 by adding acetate buffer (5 ml., 0.2N) followed by sulphuric acid (3.85 ml., 1.0N). Sodium periodate solution (5 ml., 0.05M) was then added and the solutions allowed to stand in the dark for varying intervals of time. The excess sodium periodate was estimated by the method of Miller and Friedberger [50] using 0.05M sodium arsenite solution. A blank solution was run corresponding to each of the time intervals. Moles

of periodate consumed per mole of tosyl ester : 0.0096,0.0065 (26hr); 0.037, 0.047 (90hr); 0.117,0.121 (230hr).

The action of sodium periodate on methyl 3,6-anhydro-α-D-glucoside at pH4.45.

Weighed quantities of methyl 3,6-anhydro-α-D-glucoside were dissolved in sodium hydroxide solution (10 ml.,0.4N). The solution was then neutralised and buffered as described above. When oxidised for increasing periods of time the solutions consumed 0.017, 0.018 moles (24hr); 0.071,0.076 moles (120hr); 0.13,0.12 moles (194hr.) of sodium metaperiodate per mole of 3,6-anhydro compound respectively.

The action of sodium metaperiodate at pH6.64 on the reaction product of methyl 6-O-tosyl-α-D-glucoside with excess sodium hydroxide.

20-25 mg. samples of the tosyl compound were dissolved in sodium hydroxide solution (10 ml.,0.4N) and allowed to stand overnight at room temperature. Phosphate buffer (5ml.,0.07M) was then added followed by sulphuric acid (3.95 ml.,1.0N) to adjust the pH to 6.64. The solutions were then oxidised for different periods of time with sodium metaperiodate by the method already described. The amount of periodate consumed was 0.006, 0.004 (24hr); 0.007 (120hr) and 0.006 moles (168hr) respectively. Under similar conditions methyl α-D-glucoside consumed 2.03,2.05 moles (24hr) of sodium metaperiodate.

The action of sodium metaperiodate at pH 6.64 on the reaction products of a number of methyl 6-O-tosyl-glycosides and some related compounds with excess sodium hydroxide.

When reacted with sodium hydroxide solution and oxidised with sodium metaperiodate at pH 6.64 for 24hr. as described above, methyl 6-O-tosyl-β-D-glucoside, methyl 6-O-tosyl-α and β-D-galactoside, 1,5-anhydro-6-O-tosyl-D-glucitol and 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol each consumed less than 0.002 moles of sodium metaperiodate per mole of sugar ester.

B. Attempted direct determination of the second order rate constant for the reaction of methyl 6-O-tosyl-α and β-D-glucosides with sodium hydroxide and observations on the acid dissociation of some 3,6-anhydro compounds.

The alkaline cyclisation of 6-tosyl esters to 3,6-anhydro compounds bears a formal analogy to the reaction between ethylene chlorohydrin and sodium hydroxide. This reaction is generally considered to proceed via an intermediate alkoxide ion and it has been demonstrated that, at least to a first approximation, the reaction is first-order with respect to each of the reactants[37,62]. Initially, therefore, it was hoped that the investigation of the reaction of the 6-tosylates with an equimolecular quantity of sodium hydroxide would yield second-order rate constants which would serve as a measure of the relative reactivity of the various compounds.

The rate of change of some physical property of a solution, providing it is related in some simple way to changes in concentration of the reactants or products of the reaction, offers a ready method for the determination of rate constants. Walker and Kay[63] and many subsequent workers have made use of changes in the conductivity of solutions for this purpose.

For a second-order reaction between equivalent concentrations of reactants, the rate equation is $kt = \frac{x}{a(a-x)}$ where k is the second-order rate constant for the reaction, t is time, a is the initial concentration of the reactants and

x is the fraction reacted in time t.

Assuming a linear relationship between conductivity and concentration for dilute solutions, the rate equation can be written

$$t = \frac{1}{ak}\left[\frac{C_o - C_\infty}{C_x - C_\infty}\right] - \frac{1}{ak}$$, where C_o is the initial conductivity of the solution, C_∞ the final conductivity and C_x the conductivity after a time t. Hence a plot of $\frac{1}{C_x - C_\infty}$ against t is a straight line of intercept $\frac{1}{C_o - C_\infty}$ and slope $\frac{ak}{C_o - C_\infty}$.

In the present work a series of preliminary experiments was carried out in which methyl 6-O-tosyl α-D-glucoside and sodium hydroxide were reacted at approximately 0.01M concentrations in a conductivity cell kept at 40.0° by means of a thermostatically controlled water bath. The fall in conductivity of the solution was measured at fixed intervals and the results plotted according to the usual second-order rate equation. Linear plots were obtained, although the results for duplicate experiments were not in very good agreement. Further experiments were carried out in which the amount of sodium hydroxide remaining was determined at fixed intervals of time by pipetting aliquot portions of the solution into a standard volume of acid and titrating the excess of acid with a standard solution of sodium hydroxide; good linear second-order plots were obtained in this way for methyl 6-O-tosyl-α-D-glucoside, but an experiment using methyl 6-O-tosyl-β-D-glucoside showed some

deviation from linearity in the later stages of the reaction (see P.61).

Unfortunately the classical second-order rate constants obtained by the two methods were not in good agreement. Even when the two methods were used simultaneously in the same run, the half-life for the reaction of methyl 6-O-tosyl-α-D-glucoside was 106 minutes when measured by conductivity, whereas the acid-alkali titration method gave a half-life of 165 minutes. The rate constants are compared in table 1.

T A B L E.1.

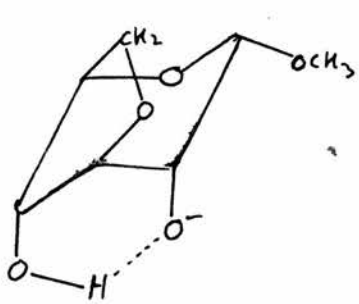
Compound	Rate by conductivity (litre moles^{-1}sec^{-1})	Rate by acid-alkali (litre moles^{-1}sec^{-1})
Methyl 6-O-tosyl-α-D glucoside.	1.73×10^{-2} 1.84×10^{-2}	1.06×10^{-2} 1.08×10^{-2}
Methyl 6-O-tosyl-β-D glucoside.	1.23×10^{-2}	0.72×10^{-2}

One possible explanation for the surprising discrepancy between the results is that the 3,6-anhydro compound produced is sufficiently acidic to be appreciably ionised thus removing hydroxyl ions and giving an inaccurate result when the conductivity method is used. An investigation of the effect of methyl 3,6-anhydro-α-D-glucopyranoside on the conductivity of dilute sodium hydroxide solution confirmed that the 3,6-anhydro compound is appreciably

ionised as shown by the considerable fall in conductivity of the solution (see Table 2. P.47). Other 3,6-anhydro-glycopyranosides were also found to depress the conductivity of sodium hydroxide solutions but this was not universally the case. Whilst methyl 3,6-anhydro- α and β -D-glucopyranoside and 1,5:3,6-dianhydro-D-glucitol produced marked changes in conductivity, methyl 3,6-anhydro- α -D-mannoside and methyl 3,6-anhydro - α -D-galactoside gave only small depressions. 1,5:3,6-dianhydro-2-deoxy -D-glucitol showed a somewhat larger depression than the galactose or mannose derivatives but, owing to the very hygroscopic nature of this compound, the accuracy of the measurement is in some doubt.

A consideration of the possible conformations of the 3,6-anhydro compounds shows that the 1C conformations of those which produce large depressions in conductivity are those having a 1,3-diaxial arrangement of hydroxyl groups. In the less acidic compounds the hydroxyl groups have an a:e disposition.

An explanation of the enhanced acidity of some of the 3,6-anhydro compounds in terms of intramolecular hydrogen bonding is suggested. It seems probable that, in sodium hydroxide solution at least, the methyl 3,6-anhydro- α and β -D-glucosides and 1,5:3,6-dianhydro-D-glucitol exist in the 1C conformation and that the high acidity of these compounds is due to stabilisation by hydrogen bonding of the anion produced by ionisation of either the 2 or 4 hydroxyl group.



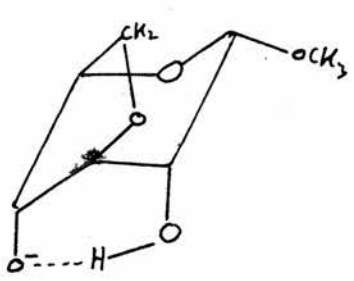
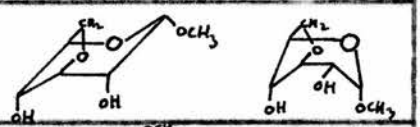
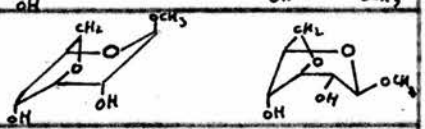
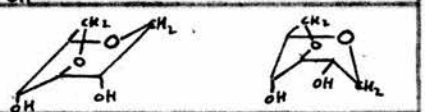
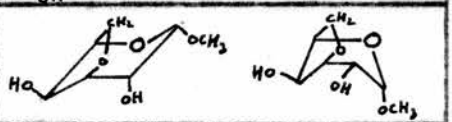
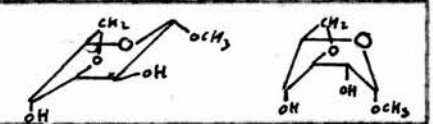
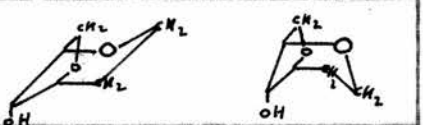
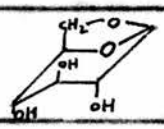
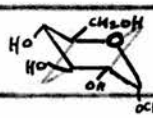
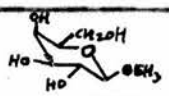
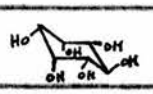
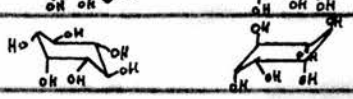


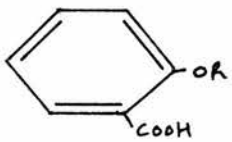
TABLE 2.

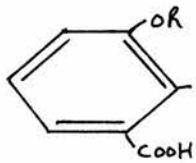
The effect of 0.01M concentrations of anhydro sugars on the conductivity of 0.01N sodium hydroxide solutions at 40.00 ± 0.02°

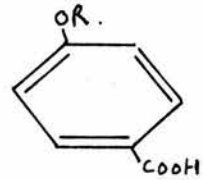
Compound added	% Fall in conductivity	Possible conformations of anhydro sugars
Methyl 3,6-anhydro-α-D-glucopyranoside	19.5	
Methyl 3,6-anhydro-β-D-glucopyranoside	23.5	
1,5:3,6-dianhydro-D-glucitol	18.8	
Methyl 3,6-anhydro-α-D-galactopyranoside	3.9	
Methyl 3,6-anhydro-α-D-mannopyranoside	3.9	
1,5:3,6-dianhydro-2-deoxy-D-glucitol	3.8	
Methyl 3,6-anhydro-α-D-glucofuranoside	1.35	
1,6-anhydro-D-glucose	2.2	
1,4-anhydro-D-mannitol	4.5	
Sucrose	8.4	
Methyl α-D-glucoside	2.2*	
Methyl β-D-galactoside	1.85*	
Epi-Inositol	2.1	
Myo-Inositol	1.74	

* at 20.0° C.

There is ample evidence that hydrogen bonding does indeed lead to increased acidity in some compounds. This is illustrated by the observed pK values for a series of salicylic acid derivatives[64].

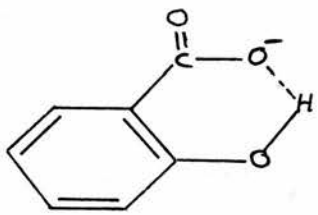






	pK_a	pK_a	pK_a
R=H	2.98	4.08	4.58
$R=CH_3$	4.09	4.09	4.47
$R=C_2H_5$	4.21	4.17	4.45

The increased acidity of salicylic acid over the other compounds is clearly due to the stabilising effect of hydrogen bonding on the anion.



In view of these considerations, a further series of compounds having cis 1,3-hydroxyl groups was examined together with several other compounds having a preponderance of hydroxyl groups in close proximity. In no case was a marked decrease in conductivity observed when the compounds were dissolved in sodium hydroxide solution. In particular the failure of 1,6-anhydro-D-glucose, shown by Reeves[65] to have a chair conformation and thus a 1,3-diaxial arrangement of hydroxyl groups, to produce a significant depression in conductivity is very puzzling. However, it is

relevant to note that molecular models (Dreiding type) suggest that in the 3,6-anhydroglucopyranosides the presence of the five-membered ring tends to force the 2-and 4-hydroxyl groups closer together than they would be if the pyran ring was undistorted; in 1,6-anhydro-D-glucose the distortion has the opposite effect.

Neither epi- nor myo- inositol produce a large decrease in the conductivity of sodium hydroxide solution under the conditions used here, even though the former has a 1,3-diaxial arrangement of hydroxyl groups. However, it seems probable that investigations using a higher concentration of alkali would reveal a difference in the acid dissociation of these compounds; in fact, Frahn and Mills[66] have shown recently that the inositols which have 1,3-diaxial hydroxyl groups migrate faster on electrophoresis in 0.1N sodium hydroxide than the isomers in which this feature is absent.

Foster, Overend and Vaughan[67] have suggested on the basis of theoretical considerations that methyl 3,6-anhydro- α-D-glucoside and -galactoside exist in the chair form but that the β-glycosides have the boat conformation. However, Table 2 shows that the α- and β-glucosides depress the conductivity of sodium hydroxide by similar amounts and, if the above interpretation of this phenomenon is correct, it seems likely that both anomers exist substantially in the chair form. Independent evidence of the conformation of the β-anomer would clearly be valuable, not only in connection with the present results but also for the interpretation of the kinetic data discussed in a later section of this thesis. A more extensive investigation of the ionisation phenomenon

would also be of interest; in particular, a study of the known methyl 3,6-anhydro-4-O-methyl-α-D-glucoside would exclude the possibility that ionisation of the hydroxyl group in the 2-position, unassisted by hydrogen-bonding, could account for the results.

As mentioned in the introduction, infrared spectroscopy provides a powerful method for the investigation of hydrogen-bonding in sugar derivatives[26] and it seemed of interest to examine a 3,6-anhydro-glucoside by this technique to see if the hydrogen-bonding between 1,3-diaxial hydroxyl groups could be demonstrated in the unionised compound. This work was kindly undertaken by Dr. G. Eglinton using a Unicam SP.100 spectrophotometer equipped with a prism-grating double monochrometer.

The spectrum of a dilute (0.0016M,2cm.cell) solution of methyl 3,6-anhydro-α-D-glucopyranoside in carbon tetrachloride showed two peaks of approximately similar intensity at 3509cm^{-1} and 3560cm^{-1} respectively. The former bond is clear evidence of a fairly strong hydrogen bond, presumably between the axial oxygens at $C_{(2)}$ and $C_{(4)}$. It is interesting that this absorption frequency is even lower than those observed by Spedding[28] for hydrogen bonds between axial oxygens in a number of 4,6-benzylidene glycosides. West,Korst and Johnson[68] have found absorption at 3500cm^{-1} for the hydrogen bond between axial oxygen atoms in the (3,3,1)-bicyclononone series (see also ref 69 for hydrogen bonding in 10-methyldecalin-2,9-diols). The position of the second bond in the spectrum of the anhydro glucoside indicates that the second hydroxyl is also hydrogen-bonded, but the bond is weaker and its position cannot be assigned with certainty. No absorption was observed in the region

corresponding to free hydroxyl groups ($\simeq$ 3600cm^{-1}).

The spectra of methyl 3,6-anhydro- α-D-galactopyranoside and α-D-mannopyranoside were also examined; owing to the low solubility of these compounds in carbon tetrachloride, chloroform had to be used as solvent. In this solvent the glucoside absorbed at 3490 and 3552cm^{-1}, the extinction coefficients being independent of concentration in the region $\simeq$ 0.005M, showing the absence of intermolecular hydrogen-bonding. Under the same conditions, the mannoside and galactoside gave peaks at 3612 and 3613cm^{-1} respectively due to free hydroxyl groups. In addition the mannoside gave a peak at 3565cm^{-1} and the galactoside a shoulder in this region; these correspond approximately to the weaker bond in the glucoside. No absorption corresponding to the strong bond in the glucoside was observed for the galactoside or mannoside. A more extensive investigation of the infrared spectra of 3,6-anhydro compounds would obviously be of interest.

Returning to the kinetics, it is clear that the ionisation of the 3,6-anhydro compounds not only invalidates the conductometric determination of the extent of reaction but will also affect the kinetic form, since the ionisation of the product depletes the hydroxide concentration. An attempt was made to calculate the kinetic consequences of this ionisation but the resulting equation was very complicated and this approach was abandoned as unprofitable. An alternative course is to study the reaction using a large excess of sodium hydroxide; work on these lines is described in the next section.

EXPERIMENTAL.

Preparation of carbonate-free sodium hydroxide solutions.

The sodium hydroxide used for the conductivity measurements was obtained by percolating an approximately 0.02N solution of analytical reagent quality sodium chloride through a column of IRA-400 anion exchange resin. It has been claimed that in this way sodium hydroxide free from contamination by atmospheric carbon dioxide is obtained[70]. In the present work, although a closed system was used, the sodium hydroxide solution, when titrated with hydrochloric acid showed a discrepancy between phenolphthalein and methyl red indicator end-points representing some 2-3% of sodium carbonate. It is thought that this was due to contamination of the solution with traces of amino compounds from the resin, since after long contact with resin, the solutions smelled strongly of amines. The sodium hydroxide solution was generated as required; the conductivity of the solutions obtained in this way, after dilution to the appropriate strength with de-ionised water, differed by less than 1% on average.

Determination of reaction rate by conductivity method.

Apparatus:-

A conductivity cell was constructed having two separate cylindrical glass vessels each of approximately 40 ml. capacity and joined by a centre tube which permitted rapid mixing of the solutions contained in the two halves of the cell. Into one arm of the cell was inserted the electrode, consisting of ca. 1 cm. square pieces of heavily platinised platinum foil rigidly fixed ca. 1 cm. apart. The cell constant was found to be 0.3256, using 0.01M potassium chloride at $24.97^{\circ} \pm 0.02^{\circ}$.

The electrode was sealed into a Quickfit ground glass stopper, and the remaining half of the cell was also sealed by means of a ground glass stopper.

Conductivity measurements were made by means of a Cambridge conductivity bridge L.76294, which consists of an alternating current Wheatstone Bridge operated from a 1,000 cycles per second Cambridge Reed Hummer. The nullpoint was determined by means of a tuned headphone.

Experimental procedure:-

The air in the conductivity cell was displaced by nitrogen which was freed from carbon dioxide by passing first through sodium hydroxide solutions and then up a tower packed with soda lime.

20 ml. of a standard solution of sodium hydroxide were run from the burette of the apparatus for the direct preparation of carbonate-free sodium hydroxide into one arm of the conductivity cell and the stopper replaced.

20 ml. of a standard solution of methyl 6-O-tosyl-α-D glucoside was then pipetted into the second arm of the cell, the flow of nitrogen was stopped and the electrode inserted immediately. The cell was then immersed in a water bath at $40.00 \mp 0.02^{\circ}$, the temperature being maintained by means of a "Tempunit" thermostat (Techne, Cambridge).

When thermal equilibrium was reached, the two solutions were mixed rapidly by tilting the cell several times to ensure complete homogeneity, and conductivity measurements made at frequent intervals. The final reading (C_{∞}) was obtained by keeping the solution at 40.00° until the conductivity was essentially constant over a period of 48 hours.

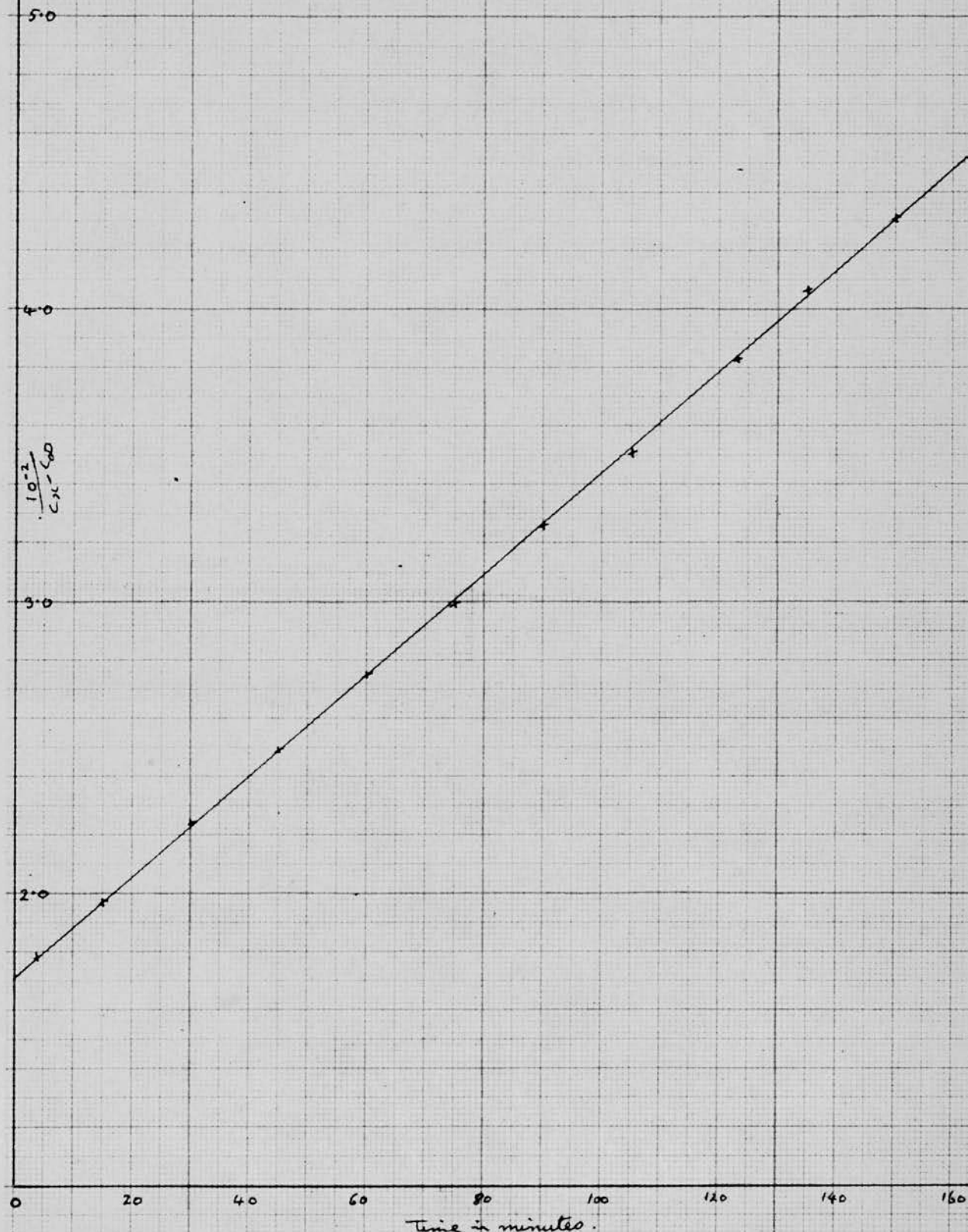
The typical plot shown on page 55 was obtained using the data in Table 3.

TABLE 3.

Typical data for the reaction of methyl 6-O-tosyl-α-D-glucoside 0.01M with sodium hydroxide 0.01N at 40.00 ± 0.02° : conductivity method.

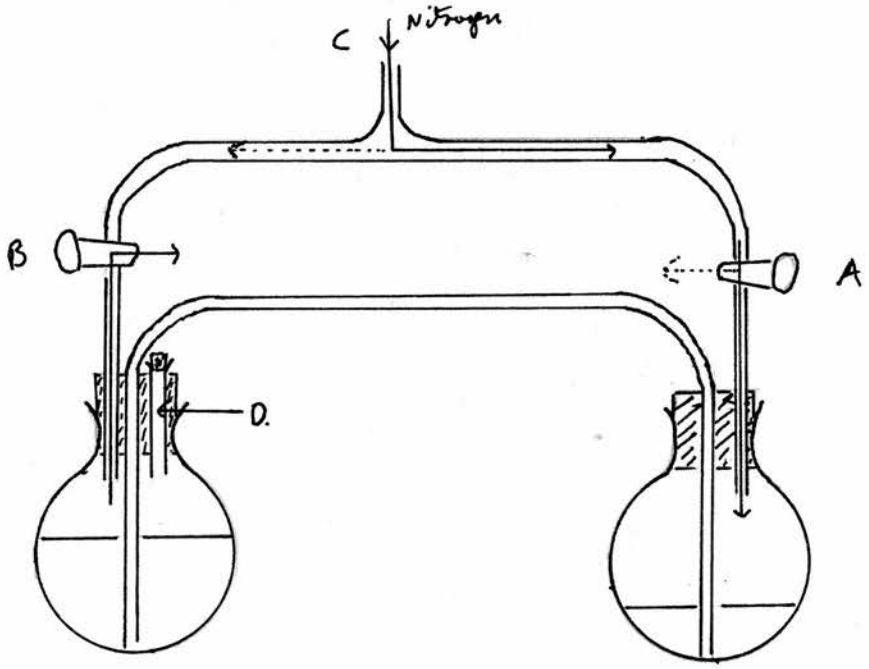
Time in mins.	$10^2 C_x$	$10^2(C_x - C_\infty)$	$\frac{10^{-2}}{C_x - C_\infty}$
0	-	-	-
2	0.898	0.562	1.78
15	0.833	0.507	1.97
30	0.772	0.446	2.24
45	0.727	0.401	2.49
60	0.690	0.364	2.75
75	0.659	0.333	3.00
90	0.633	0.307	3.26
105	0.611	0.285	3.51
121.5	0.582	0.261	3.83
135	0.572	0.246	4.07
150	0.558	0.232	4.31
165	0.544	0.218	4.59
180	0.533	0.207	4.83
∞	0.326	-	-

Rate of reaction of methyl 6-O-tosyl-α-D-glucoside (0.01M) with sodium hydroxide (0.01N) at 40.00 + 0.02° : conductivity method



Determination of the reaction rate by acid-alkali titration.

APPARATUS.



Method:-

Nitrogen was addmitted to the apparatus at C and allowed to flow out at B. After complete displacement of the air, 60 ml. of an approximately 0.02N solution of sodium hydroxide were run in through D from the apparatus for the continuous production of carbonate-free sodiun hydroxide. Two 5 ml. samples were then withdrawn by means of a pipette and titrated under nitrogen with 0.01N solution of sulphuric acid using methyl red as indicator.

An amount of 6-tosyl ester equivalent to the sodium hydroxide remaining in the first flask was transferred to the second flask by the use

of 50 ml. of deionised water from a pipette. The flask was disconnected from the remainder of the apparatus during this operation, and following it, the air in the flask was displaced by nitrogen and the apparatus reassembled. The solutions were allowed to come to thermal equilibrium in the thermostat tank and then mixed rapidly by reversing the flow of nitrogen through the apparatus several times; this operation could be completed in 30 seconds.

5 ml. samples were then removed at 15 minute intervals by means of a pipette and transferred to 10 ml. of 0.01N sulphuric acid solution to stop the reaction. The excess of sulphuric acid was then back titrated with approximately 0.01N sodium hydroxide solution from a microburette using methyl red as indicator. The sodium hydroxide solution was standardised by titration against the 0.01N sulphuric acid used in the experiment.

Plots from typical sets of data obtained for methyl 6-O-tosyl-α-D-glucoside (Table 4) and methyl 6-O-tosyl-β-D-glucoside (Table 5) are shown on pages 59 and 61 respectively. The deviation from linearity of the plot for methyl 6-O-tosyl-β-D-glucoside is reflected in the gradual decrease of the value of $\frac{1}{t}\left(\frac{1}{a-x}-\frac{1}{a}\right)/k_2$ (shown in column 6 of Table 5).

Determination of the conductivity of some anhydro sugars in sodium hydroxide solution.

Carbonate-free sodium hydroxide solution (30 ml. approx. 0.01N) was introduced into one arm of the conductivity cell used for the rate determinations. The amount of anhydro sugar required to give a 0.01N

TABLE 4.

Typical data for the rate of reaction of methyl 6-O-tosyl-α-D-glucoside (0.0098M) in sodium hydroxide (0.00987N) at 40.00 ± 0.02° determined by acid-alkali titration.

Time in minutes	Back titre ml 0.00987N NaOH	$(a - x)$ †	$\frac{1}{a - x}$	$\frac{1}{a-x} - \frac{1}{a}$	$10^2 k_2$ Litre$_1$ moles^{-1} sec^{-1}
0	5.14 *	5.00	0.197	-	-
15	5.60	4.54	0.220	0.023	1.304
30	5.98	4.16	0.240	0.043	1.066
45	6.30	3.84	0.260	0.063	1.191
60	6.60	3.54	0.283	0.086	1.219
75	6.81	3.33	0.300	0.103	1.170
90	7.00	3.14	0.319	0.122	1.153
105	7.20	2.94	0.340	0.143	1.158
120	7.32	2.82	0.355	0.158	1.119
135	7.46	2.68	0.373	0.173	1.090
150	7.58	2.56	0.391	0.194	1.100
165	7.69	2.45	0.408	0.211	1.087
180	7.81	2.33	0.429	0.232	1.096
195	7.90	2.24	0.446	0.249	1.088
210	7.97	2.17	0.461	0.264	1.069
225	8.09	2.05	0.488	0.291	1.100

* Obtained from standardisation.

† 5 ml. = 0.00987 mols/litre.

Rate of reaction of methyl 6-O-tosyl-α-D-glucoside (0.0098M) with sodium hydroxide (0.00987N) at 40.00 ± 0.02° ; determined by acid-alkali titration

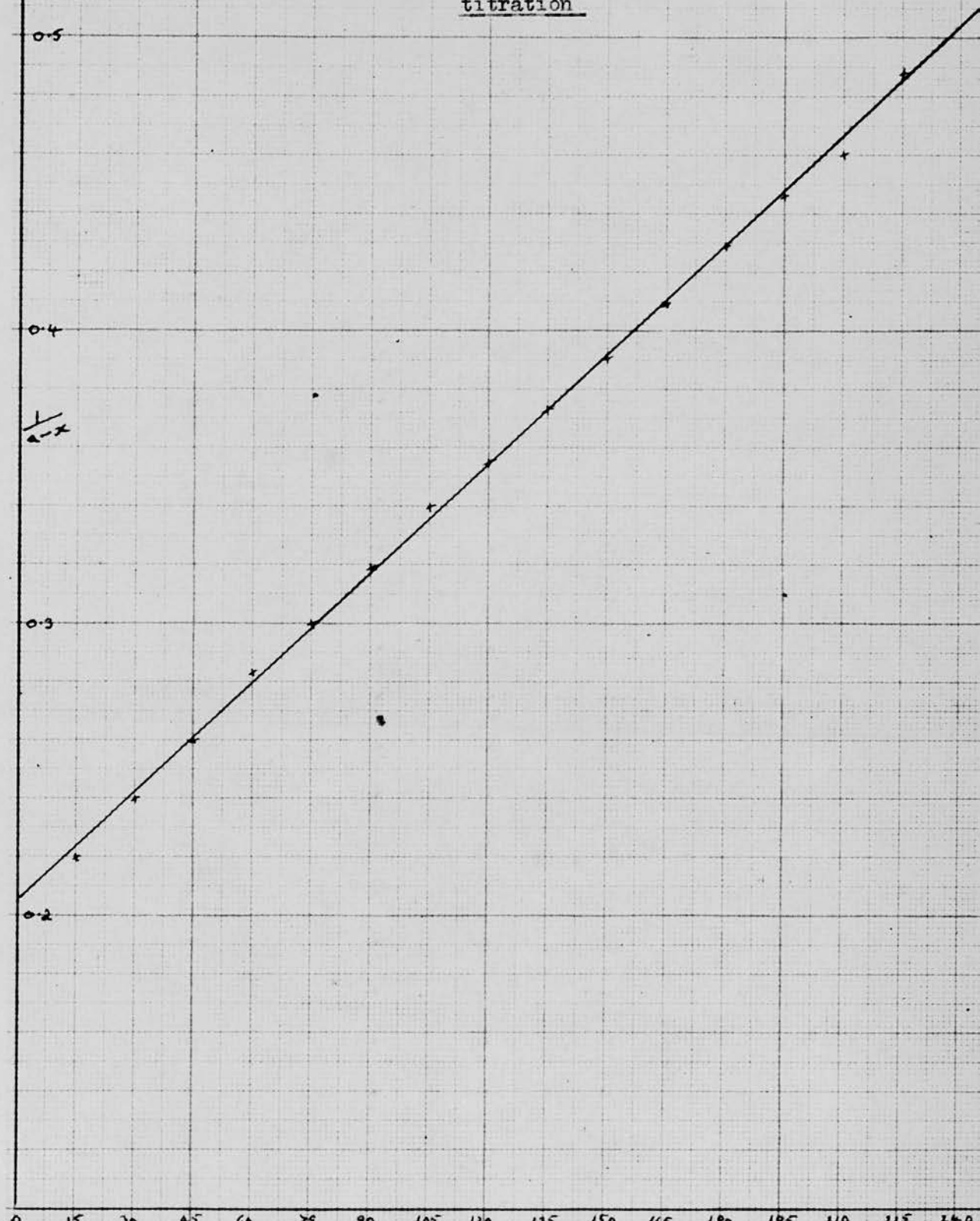


TABLE 5.

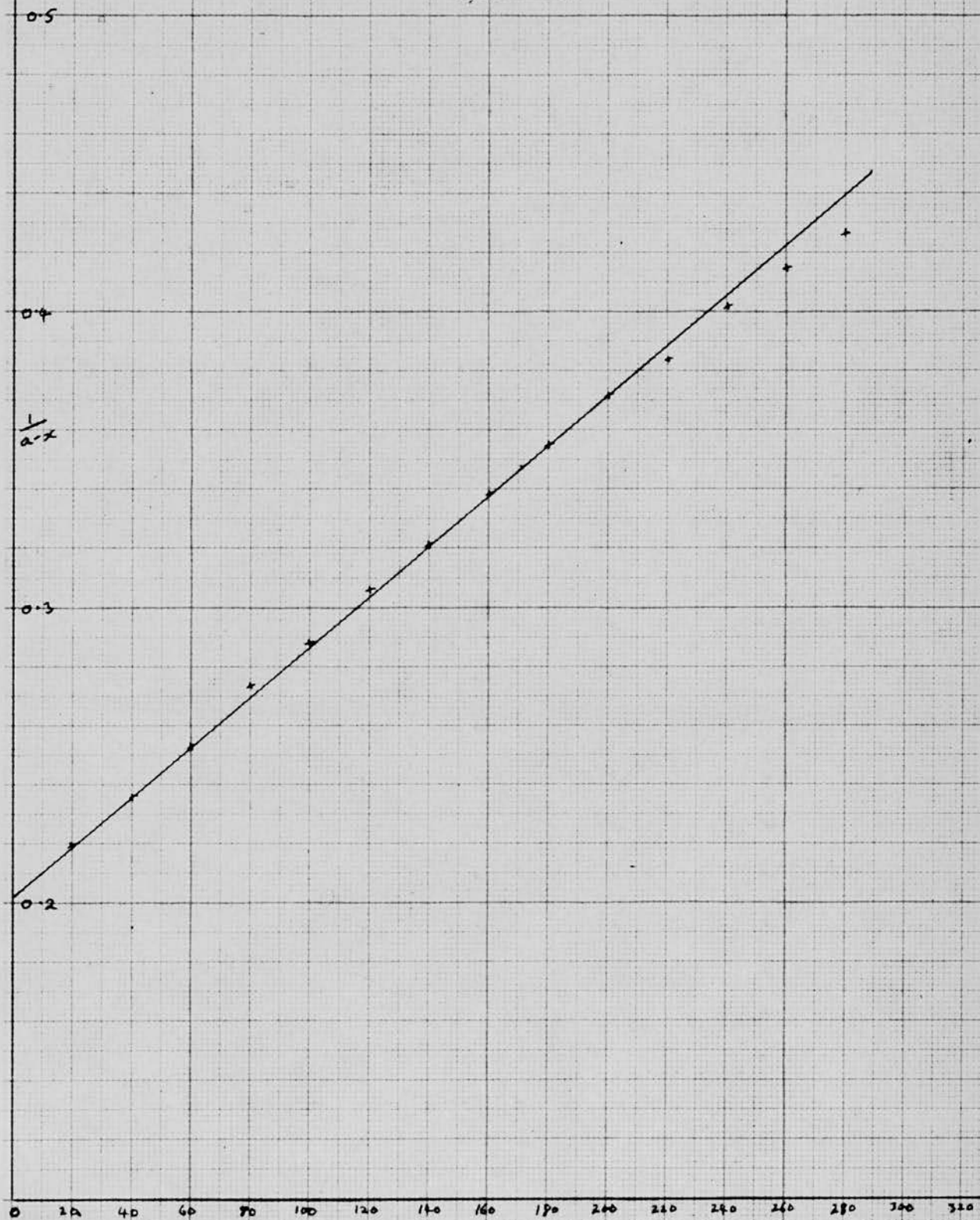
Typical set of data for the rate of reaction of methyl 6-O-tosyl-β-D-glucoside (0.01M) with sodium hydroxide (0.01014N) at 40 ± 0.02°.

Time in minutes	Back titre ml 0.00997N NaOH	a - x †	$\frac{1}{a-x}$	$\frac{1}{a-x} - \frac{1}{a}$	$10^2 k_2$ litre moles^{-1}sec^{-1}
0	(4.95)*	5.08 *	0.1994	-	-
20	5.48	4.55	0.2198	0.0204	0.6517
40	5.80	4.23	0.2364	0.0370	0.7725
60	6.09	3.94	0.2538	0.0544	0.7571
80	6.38	3.65	0.2740	0.0746	0.7787
100	6.56	3.47	0.2882	0.0888	0.7415
120	6.76	3.27	0.3058	0.1064	0.7404
140	6.92	3.11	0.3215	0.1221	0.7283
160	7.08	2.95	0.3390	0.1396	0.7286
180	7.22	2.81	0.3559	0.1565	0.7261
200	7.34	2.69	0.3717	0.1723	0.7194
220	7.43	2.60	0.3846	0.1852	0.7030
240	7.54	2.49	0.4016	0.2022	0.7036
260	7.62	2.41	0.4149	0.2155	0.6921
280	7.69	2.34	0.4274	0.2280	0.6800

* Calculated from standardisations.

† 5.08 ml = 0.01014 mol/litre.

Rate of reaction of methyl 6-O-tosyl-β-D-glucoside (0.01M) with sodium hydroxide (0.0101N) at 40.00 ± 0.02° : determined by acid-alkali titration



concentration when dissolved in 30 ml. of solution, was placed in the second arm of the cell, the operations being done in an atmosphere of nitrogen to prevent absorption of carbon dioxide. The cell was sealed and placed in a water bath, the temperature of which was kept at 40.00 ± 0.02°C by means of a thermostat, and maintained until the conductivity of the sodium hydroxide solution became constant. The cell was then inverted several times to dissolve the anhydro compound and the conductivity redetermined, when again constant. The results are given in Table 6.

Because of their importance in the interpretation of the kinetics a careful comparison of the effect of methyl α-D-glucoside and methyl β-D-galactoside on the conductivity of sodium hydroxide solutions was made. The compounds were crystallised several times from water to remove any inorganic material which would effect the result. The increase in resistance of approx. 0.01N sodium hydroxide solution on addition of the appropriate glycoside was measured for various concentrations of added sugar. The experimental procedure was as described for the anhydro compounds but the measurements were made at 19.95° using a Tinsley Type 4896 conductivity bridge. The experimental results are shown in Table 7.

TABLE 6.

Compound added	Conc'n. of sugar (M)	Initial conductivity of NaOH sol'n. 10^{-2} mhos.	Conductivity of alkaline sugar sol'n. 10^{-2} mhos
Methyl 3,6-anhydro-α-D-glucopyranoside	0.01	0.935	0.753
Methyl 3,6-anhydro-β-D-glucopyranoside	0.01	0.915	0.700
1,5:3,6-dianhydro-D-glucitol	0.01	0.908	0.737
Methyl 3,6-anhydro-α-D-galactopyranoside	0.01	0.922	0.886
Methyl 3,6-anhydro-α-D-mannopyranoside	0.01	0.919	0.883
1,5:3,6-dianhydro-2-deoxy-D-glucitol	0.01	0.912	0.877
Methyl 3,6-anhydro-α-D-glucofuranoside	0.005	0.902	0.890
1,6-anhydro-D-glucose	0.01	0.919	0.899
1,4-anhydro-D-mannitol	0.01	0.913	0.872
Sucrose	0.01	0.916	0.839
Epi-Inositol	0.01	0.911	0.892
Myo-Inositol	0.01	0.918	0.902

TABLE 7.

Compound	Conc'n. of Sugar.	Resistance in ohm's of sodium hydroxide solution.	Resistance in ohm's of sodium hydroxide solution and sugar.	Increase in resistance in ohm's.
Methyl α -D-glucoside,	0.05N	153.0	167.4	14.1
	0.1N	153.1	181.7	28.6
	0.2N	153.6	207.3	53.7
Methyl β -D-galactoside.	0.05N	153.4	168.1	14.7
	0.1N	152.6	179.3	26.7
	0.2N	154.0	205.0	51.0

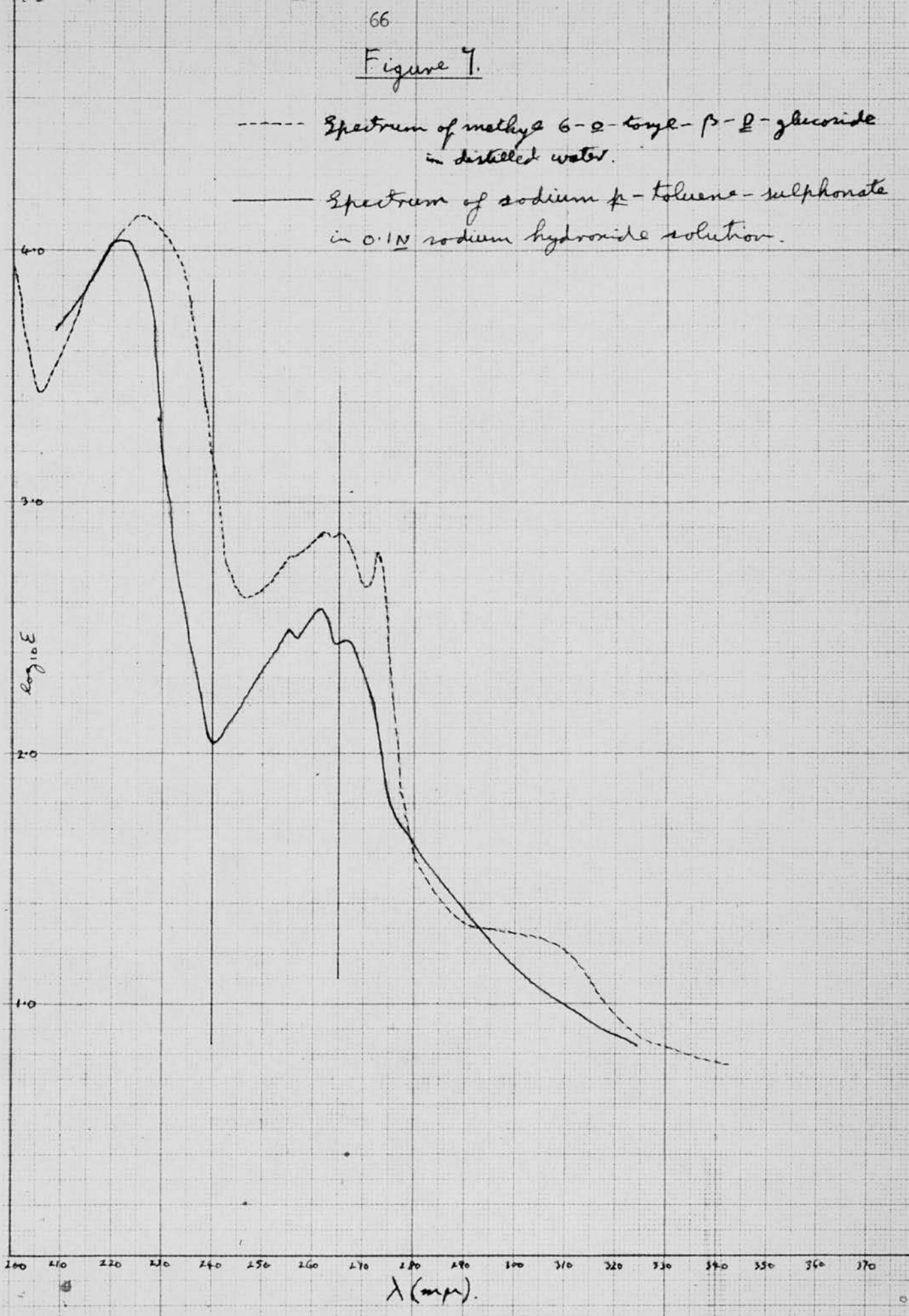
C. Determination of first-order rate constants using a large excess of sodium hydroxide.

1) Preliminary experiments.

Many of the difficulties involved in the measurement of the second-order rate constants for the reaction between the 6-tosyl esters and sodium hydroxide are eliminated if a large excess of sodium hydroxide is used when the reaction becomes first-order. Dividing the observed first-order rate constant by the hydroxide concentration should thus give the second-order rate constant, provided that the reaction follows a ~~simple~~ simple bimolecular scheme. Since in presence of a large excess of alkali conductivity and acid-alkali titration methods are unsuitable for rate determination and alternative procedure was devised.

A comparison of the ultraviolet absorption spectra of solutions of sodium tosylate and methyl 6 -O-tosyl- β -D-glucoside (Fig.7.) shows that these are sufficiently different to permit changes in optical density during the reaction to be used for rate measurements. It was assumed that changes in concentration of the reactants and products were linear with respect to changes in optical density in accordance with Beer's law. Exploratory experiments were done in which the rate of decrease of optical density at 265 mμ of a 0.001M solution of methyl 6-O -tosyl- α -D-glucoside in 0.05N sodium hydroxide (a fiftyfold excess) was measured in a 1 cm. silica cell, using a Unicam SP. 500 spectrophotometer. The optical density was determined at fixed intervals over 3-4 half-lives of the reaction and a final reading obtained by

Figure 7.


------ Spectrum of methyl 6-o-tosyl-β-D-glucoside in distilled water.
——— Spectrum of sodium p-toluene-sulphonate in 0·1N sodium hydroxide solution.
4·0
3·0
2·0
1·0
log10 E
200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370
λ (mμ).

allowing the solution to remain in the instrument until the reaction was essentially complete (ca.10 half-lives).

The total change in optical density was approximately 0.5 units. Satisfactory linear plots were obtained when the experimental data was plotted using the classical first-order rate equation which for changes in optical density may be expressed as:-

$$kt = 2.303 \log \frac{E_o - E_\infty}{E_t - E_\infty}$$ where E_o, E_∞ and E_t are the initial and final optical density and the value after time t respectively. Details and typical data are given in the experimental section.

When using higher concentrations of sodium hydroxide a slight increase in the absorption of the solution was observed near the end of the reaction. The increase was usually of the order of 2% of the total change in optical density and for evaluation of the rate constant the minimum observed value was used; in most cases this was reached after 10-12 half-lives. An investigation of the effect of sodium hydroxide on the absorption of methyl 3,6-anhydro-α-D-glucopyranoside, which itself does not absorb in the ultraviolet region, and of the effect of carbon dioxide on the absorption spectrum of sodium hydroxide failed to reveal the cause of the increase.

The increase, although slight, was disconcerting and, as a check on the validity of the results the rate constants were also calculated by the method introduced by Guggenheim[71]. Readings of the optical density were made at fixed intervals of time, and after approximately two half-lives of the reaction a second series of readings was commenced at similar time intervals, or more usually, since the change in optical density in a given

time was now much smaller, at twice the time interval, the intermediate readings being obtained by graphical interpolation. Guggenheim showed that for a first-order reaction a plot of t against $\log \frac{1}{E_t - E_{(t+r)}}$ is linear with slope equal to $\frac{k}{2.303}$. Usually the interval r was chosen to represent between $1\frac{1}{2}$-2 half-lives of the reaction. This method is independent of the final value of the optical density of the solution.

In the present work the rate constants were evaluated by the end value method and by the method of Guggenheim for each experiment. Both methods were usually found to give linear first-order plots up to 3 or 4 half-lives; occasionally deviation from linearity did occur when using the end value method, particularly in cases where there was a rise in absorption at the end of the reaction. The rate constants obtained by the two methods showed good agreement (usually within 2%). The reproducibility between duplicate runs was generally within 5%. The general procedure was considered to be satisfactory and a comprehensive series of rate determinations was carried out using the 6-tosyl esters described in Part I.

2) Salt effects and the stability of the 6-O-tosyl compounds in 3M sodium chloride solution.

Preliminary determinations of the rate of reaction of methyl 6-O-tosyl -α-D-glucoside with a range of concentrations of sodium hydroxide solution showed that the derived second-order rate constant $(\frac{k}{[OH^-]})$ increased markedly with increasing concentrations of sodium hydroxide. Ionic strength effects were suspected and a cursory investigation of the effect of sodium chloride on the reaction rates of the 6-tosyl esters of sugars

with sodium hydroxide was carried out. Rate determinations were made at two different concentrations of sodium hydroxide in most cases and the rates compared with those obtained when the ionic strength of the solution was raised to 3M by the addition of sodium chloride. In the case of methyl 6-O-tosyl-α-D-glucoside no appreciable salt effect was observed, although in 0.05N sodium hydroxide solution the rate was marginally faster in the absence of salt. For methyl 6-O-tosyl-β-D-glucoside, methyl 6-O-tosyl α and β-D-galactosides, 1,5-anhydro-6-O-tosyl-D-glucitol and its 2-deoxy analogue there was a decrease in the rate of reaction of the order of 20% when the ionic strength was raised to 3M. The experimental results are summarised in Table 8. In this and all subsequent tables the first-order rate constants are given in sec^{-1} and second-order rate constants in litre $moles^{-1}$ sec^{-1}. The symbols k^{G} and k^{∞} are used to indicate rate constants derived by the Guggenheim method and by the use of an experimental end value respectively.

The reaction between the 6-tosyl ester of a sugar and sodium hydroxide bears a formal analogy with the reaction between ethylene chlorohydrin and alkali. Warner and his co-workers[72] have demonstrated that for this reaction there is no significant salt effect at low hydroxide concentrations, as would be expected for the reaction between a neutral molecule and an ion. As a result of a more recent investigation, Ballinger and Long[73] suggest that the deviations from linearity observed at higher concentrations may largely be due to salt effects. Both in the case of ethylene chlorohydrin and for the present reactions, the charge distribution in the transition state is more diffuse than in the hydroxide ion and a slight

negative salt effect is therefore not unexpected. The result obtained for methyl 6-O-tosyl-α-D-glucoside however remains unexplained.

TABLE 8.

Salt effects +.

Compound	Conc'n of sugar ester	Conc'n of NaOH	10^5k at ionic strength 3M	10^5k in absence of NaCl
Methyl 6-O-tosyl-α-D-glucoside.	0.001M	0.05N	7.45	8.04
"	"	"	7.18	7.98
"	"	"	7.33	8.19
"	"	"	7.52	7.93
"	"	0.4N	130	129
"	"	"	132	127
Methyl 6-O-tosyl-β-D-glucoside.	"	0.4N	63.2 *	85.2 *
"	"	"	64.6 *	83.2 *
"	"	"	66.3 *	-
1,5-Anhydro-6-O-tosyl-D-glucitol.	"	0.4N	778	1003
"	"	"	783	833
"	"	"	772	-
1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol	"	0.05N	46.8	76.3
"	"	"	47.2	75.0
"	"	0.4N	372	510
"	"	"	370	502
Methyl 6-O-tosyl-α-D-galactoside.	"	0.4N	269	358
"	"	"	278	346
"	"	"	267	-
Methyl 6-O-tosyl-β-D-galactoside.	"	0.05N	27.5	41.8 *
"	"	"	27.2	41.3 *
"	"	"	26.8	-
"	"	0.4N	205	279
"	"	"	204	268
"	"	"	204	272

* k^G

+ The rates were measured at 20.04±0.03°.

As a precaution the stability of the 6-tosyl esters in 3M sodium chloride solution was determined approximately for each substance. Solutions of each of the compounds in 3M sodium chloride showed a significant decrease in optical density (at 265 mμ) on prolonged standing at 21 ± 1° but the change in most cases was too slow for the rate constants to be determined (see Table 10 page 102). In no case was the rate of change in absorption sufficiently rapid to give rise to significant errors in the rate constants for the reaction with sodium hydroxide. 1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol and, rather surprisingly, methyl 6-O-tosyl-β-D-galactoside were much the least stable of the compounds examined and the former was shown to be equally unstable in aqueous solution at this temperature (Table 9, page 101).

The order of stability of the galactoside anomers in 3M sodium chloride solution is inverted with respect to the stability of the compounds towards sodium hydroxide solution as shown by the rate constants given later. The solvolysis reaction in sodium chloride or aqueous solution may be worthy of a more thorough investigation but severe practical difficulties exist because of the slowness of the reactions.

3) Determination of the first-order rate constants for the reaction of the 6-tosylates with sodium hydroxide at various concentrations.

The first-order dependence of the reaction rate of methyl 6-O-tosyl-α-D-glucoside with sodium hydroxide on concentration of the sugar ester was confirmed. Variation of the concentration of the sugar ester between the limits 0.005 - 0.0005M showed no appreciable effect on the rate constant determined for 0.4N sodium hydroxide solutions (Table 11).

For each of the tosyl esters, rate constants were determined at five different concentrations of sodium hydroxide using at least a twentyfold excess of alkali. A minimum of two, usually three, measurements were made at each concentration. First-order rate constants were derived in each case; the results are shown in Tables 11 - 16. Good linear plots were obtained when either the method of Guggenheim (Plot of t against $\log \frac{1}{E_t - E_{(t+r)}}$) or the end value method (Plot of t against $\log \frac{1}{E_t - E_\infty}$) was used.

Except for 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol the observed pseudo first order rate constants (k) were not directly proportional to the hydroxide ion concentration. The value of the derived apparent second-order rate constants $\frac{k}{[OH^-]}$ for methyl 6-O-tosyl-α-D-glucoside, methyl 6-O-tosyl-β-D-glucoside and 1,5-anhydro-6-O-tosyl-D-glucitol showed a marked increase with increasing concentrations of sodium hydroxide (Tables 11-13). The second-order rate constants for the α and β anomers of methyl 6-O-tosyl-D-galactoside showed a small decrease with increasing hydroxide ion concentration (Tables 14 and 15), whilst for 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol, $\frac{k}{[OH^-]}$ was constant within experimental error (Table 16).

TABLE 11.

The rate of reaction of methyl 6-O-tosyl-α-D-glucoside with sodium hydroxide solution at 20.04° ± 0.03°

Conc'n of Tosyl cpd.	Conc'n. of NaOH	Ionic Strength	$10^5 k^G$	$10^5 k^\infty$	$\frac{10^4 k^G}{[OH^-]}$
0.001M	0.02N	3M	2.37	2.35	11.9
"	"	"	2.27	2.24	11.4
"	"	"	2.25	2.23	11.3
"	0.05N	"	7.93	7.45	15.9
"	"	"	7.56	7.13	15.1
"	"	"	7.98	7.33	16.0
"	"	"	7.42	7.52	14.8
"	0.1N	"	19.2	19.3	19.2
"	"	"	19.4	20.0	19.4
"	"	"	19.5	19.1	19.5
"	0.2N	"	49.6	50.0	24.8
"	"	"	49.6	52.5	24.8
"	"	"	50.5	52.1	25.3
"	0.4N	"	134	142	33.5
"	"	"	129	130	32.3
"	"	"	132	132	33.0
"	"	"	133	-	33.3
0.005M	0.4N	3M	133	-	33.3
"	"	"	133	-	33.3
0.0005M	0.4N	3M	132	-	33.0
"	"	"	134	-	33.5

* Measured in 2.0 mm. cells at 265 mμ

• Measured in 1 cm. cells at 240 mμ

TABLE 12.

The rate of reaction of methyl 6-O-tosyl-β-D-glucoside with sodium hydroxide solution at 20.04 ± 0.03°.

Concentration of tosyl compound	Concentration of NaOH	Ionic Strength	$10^5 k^G$	$10^5 k^\infty$	$\frac{10^4 k^G}{[OH^-]}$
0.001M	0.02N	3M	1.41	1.44	7.05
"	"	"	1.41	1.46	7.05
"	0.05N	"	4.22	4.73	8.44
"	"	"	4.31	4.83	8.62
"	0.1N	"	11.0	11.2	11.0
"	"	"	10.6	10.8	10.6
"	0.2N	"	26.9	28.0	13.5
"	"	"	26.6	27.5	13.3
"	0.4N	"	64.6	-	16.2
"	"	"	66.3	68.8	16.6
0.0001M*	"	"	63.2	62.9	15.8

* Measured at 240 mμ in 4 cm. cells.

TABLE 13.

The rate of reaction of 1,5-anhydro-6-O-tosyl-D-glucitol with sodium hydroxide at 20.04 ± 0.03°.

Concentration of tosyl ester	Concentration of NaOH	Ionic Strength	$10^4 k^G$	$10^4 k^\infty$	$\frac{10^3 k^G}{[OH^-]}$
0.001M	0.02N	3M	2.24	2.18	11.2
"	"	"	2.12	2.14	10.6
"	"	"	2.24	2.23	11.2
"	0.05N	"	6.18	6.10	12.4
"	"	"	6.18	6.07	12.4
"	0.1N	"	14.8	15.0	14.8
"	"	"	14.4	14.1	14.4
"	"	"	14.2	14.2	14.2
"	0.2N	"	33.1	33.8	16.6
"	"	"	33.4	33.3	16.7
"	0.4N	"	78.8	77.8	19.7
"	"	"	76.0	78.3	19.0
"	"	"	76.9	77.2	19.2

TABLE 14.

The rate of reaction of methyl 6-O-tosyl-α-D-galactoside with sodium hydroxide at 20.04 ± 0.03°.

Concentration of tosyl ester	Concentration of NaOH	Ionic Strength	$10^4 k^G$	$10^4 k^\infty$	$\frac{10^3 k^G}{[OH^-]}$
0.0001M	0.02N	3M	1.59	1.54	7.95
"	"	"	1.59	1.56	7.95
"	0.05N	"	3.79	3.62	7.58
"	"	"	3.68	3.78	7.36
"	"	"	3.80	3.81	7.60
"	0.1N	"	7.49	7.34	7.49
"	"	"	7.47	7.42	7.47
"	0.2N	"	14.7	14.4	7.35
"	"	"	14.4	13.9	7.20
"	0.4N	"	26.9	26.9	6.73
"	"	"	26.9	27.8	6.73
"	"	"	27.8	26.7	6.95

TABLE 15.

The rate of reaction of methyl 6-O-tosyl-β-D-galactoside with sodium hydroxide at 20.04 ± 0.03°.

Conc'n. of tosyl ester	Conc'n. of NaOH	Ionic Strength	$10^4 k^G$	$10^4 k^\infty$	$\frac{10^3 k^G}{[OH^-]}$
0.001M	0.02N	3M	1.08	1.06	5.40
"	"	"	1.12	1.12	5.60
"	"	"	1.11	1.09	5.55
"	0.05N	"	2.73	2.75	5.46
"	"	"	2.75	2.72	5.50
"	"	"	2.66	2.68	5.32
"	0.1N	"	5.36	5.35	5.36
"	"	"	5.44	5.89	5.44
"	"	"	5.31	5.43	5.31
"	0.2N	"	10.3	10.6	5.15
"	"	"	10.3	10.4	5.15
"	"	"	10.3	10.4	5.15
"	0.4N	"	20.6	20.5	5.15
"	"	"	20.5	20.4	5.13
"	"	"	20.6	20.4	5.15

TABLE 16.

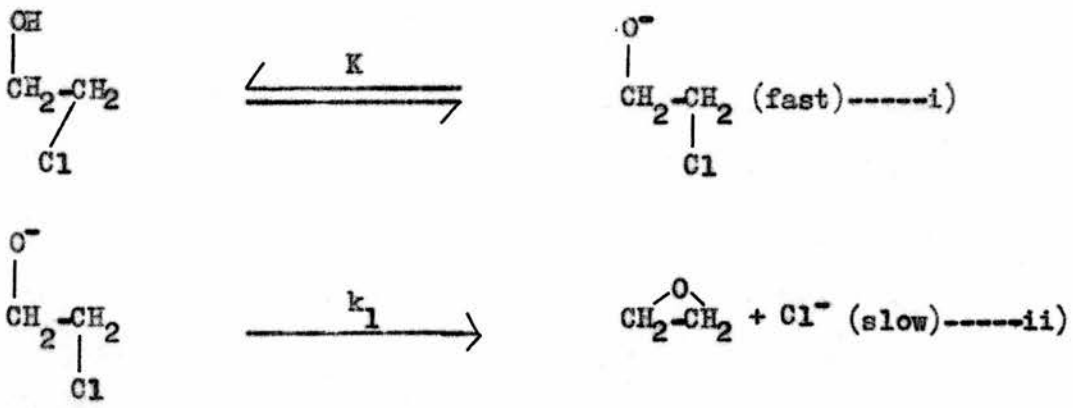
The rate of reaction of 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol with sodium hydroxide at 20.04 ± 0.03°.

Concentration of tosyl ester	Concentration of NaOH	Ionic Strength	$10^4 k^G$	$10^4 k^\infty$	$\frac{10^3 k^G}{[OH^-]}$
0.001M	0.02N	3M	1.86	1.77	9.30
"	"	"	1.89	1.88	9.45
"	"	"	1.94	1.87	9.70
"	0.05N	"	4.66	4.68	9.32
"	"	"	4.71	4.72	9.42
"	0.1N	"	9.31	9.44	9.31
"	"	"	9.44	9.39	9.44
"	0.2N	"	18.7	18.1	9.35
"	"	"	18.0	18.3	9.00
"	"	"	19.2	18.25	9.60
"	0.4N	"	35.9	37.2	8.98
"	"	"	36.8	37.0	9.20

D. Discussion of kinetic data.

1) Mechanistic aspects.

By analogy with the reaction between ethylene chlorohydrin and sodium hydroxide, the ring closure of a 6-O-tosyl-glycopyranoside to form the corresponding 3,6-anhydro derivative might be expected to involve ionic species as intermediates in the reaction. It has been demonstrated that the reaction between ethylene chlorohydrin and sodium hydroxide is first-order with respect to both of these reactants at low concentrations. Winstein and Lucas[62] proposed a two step mechanism involving a rapid ionisation equilibrium followed by a rate determining displacement of the halogen by the negatively charged oxygen as shown below:-



The rate equation of this scheme is

$$\frac{d[Cl^-]}{dt} = k_1[ClCH_2 - CH_2 - O^-] = k_1K\,[ClCH_2 - CH_2OH][OH^-]$$

If the degree of ionisation of the chlorohydrin is small, $[ClCH_2-CH_2-OH]$ can be equated with the total concentration of the chlorohydrin (unionised and ionised) and the scheme therefore leads to second-order kinetics. The observed second-order rate constant k_2 is equal to k_1K and depends upon

the acidity of the hydroxyl group (i.e. upon K). Twigg and his co-workers[74] observed some slight deviation from classical second-order kinetics when using equivalent amounts of reactants at high concentrations. This implies that ethylene chlorohydrin is appreciably ionised in concentrated alkali and that this fact cannot be neglected when deriving the rate equation. Using an approximate steady state treatment Twigg showed that a plot of $\frac{1}{k}$ against $Ao+Bo$, where Ao and Bo are the initial concentrations of the reactants and k the observed second-order rate coefficient for the early part of the reaction, was linear. From the slope and intercept it is possible to calculate both the rate constant k_2 and the equilibrium constant K for reaction 1.

In a more recent paper Ballinger and Long[73] have confirmed the presence of an appreciable amount of the alkoxide ion as an intermediate in the reaction but point out that at concentrations of sodium hydroxide of the order of 0.1N only about 5% of the initial chlorohydrin is present as the alkoxide ion. This would not result in any significant deviation from classical second-order kinetics for at least the first 50% of the reaction. However, using a conductivity method Ballinger and Long obtained a value for the acid ionisation constant of ethylene chlorohydrin in good agreement with that of Twigg.

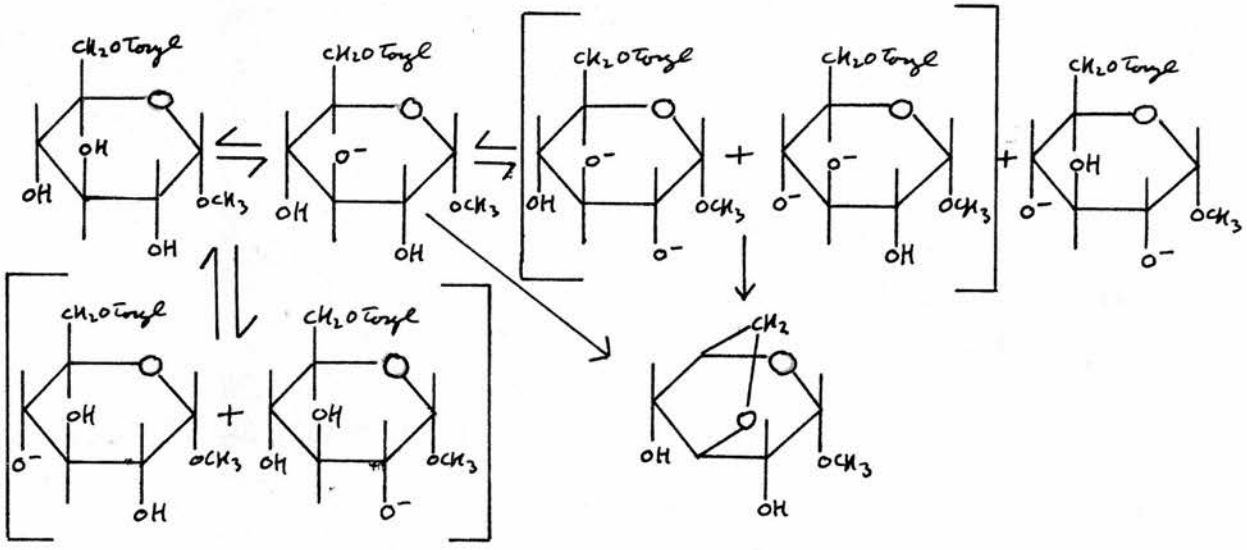
It can readily be shown that if the ionisation of the chlorohydrin is taken into account, the rate equation becomes

$$\frac{d\,[Cl^-]}{dt} = \frac{k_1 K\,[\text{total chlorohydrin}]\,[OH^-]}{1 + K\,[OH^-]}$$

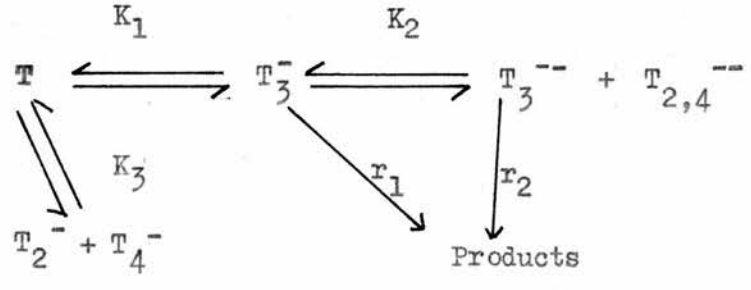
From this expression it can be deduced that the effect of an appreciable

degree of ionisation of the hydroxyl group is to produce a decrease in the observed "second-order" rate constant with increasing concentrations of hydroxide ion. Thus ionisation of the hydroxyl groups cannot account for the marked increase of $\frac{k}{[OH^-]}$ which occurs for the 6-tosyl esters of the glucose series.

In order to explain the results of the present work two assumptions were made. Firstly that, in accordance with the evidence for the ethylene chlorohydrin reaction, the sugar molecule is appreciably ionised in sodium hydroxide solution and further that, in some cases at least part of the reaction proceeds via a di-anion. Taking methyl 6-O-tosyl-α-D-glucoside as an example, the scheme shown below is envisaged.



This scheme may be represented as follows:-



where T represents the amount of unionised tosyl ester, and T_3^- and T_3^{--} are the amounts of singly and doubly ionised species respectively which lead to the formation of the 3,6-anhydro compound. T_2^- and T_4^- represent "unreactive" singly ionised species. $T_{2,4}^{--}$ represents the amount of an unreactive doubly ionised molecule present and K_1, K_2 and K_3 are the equilibrium constants for the various reactions. r_1 and r_2 are the first-order rate constants for the formation of the 3,6-anhydro compound from the singly and doubly ionised species respectively.

The equilibrium constants K_1, K_2 and K_3 for the reaction scheme are defined by the equations:-

$$K_1 = \frac{[OH^-][T]}{[T_3^-]} \quad ...i), \quad K_2 = \frac{[T_3^-][OH^-]}{[T_3^{--}]} \quad ...ii), \quad K_3 = \frac{[OH^-][T]}{[T_2^- + T_4^-]} \quad ...iii)$$

If the assumption is made that the concentration of doubly ionised species is small compared to that of singly ionised molecules, then the "total ester" concentration is given by

$$[T] + [T_3^-] + [T_2^- + T_4^-]$$

From i) and ii) $[T_3^-] = \frac{[OH^-][T]}{K_1}$, $[T_2^- + T_4^-] = \frac{[OH^-][T]}{K_3}$

so that tostal ester $= [T] + [T][OH^-](\frac{1}{K_1} + \frac{1}{K_3}) = [T](1 + Z[OH^-])$ ...iv)

where $Z = \frac{1}{K_1} + \frac{1}{K_3}$.

The overall rate equation for the reaction is given by

$$\text{rate} = r_1[T_3^-] + r_2[T_3^{--}].$$

From ii) and iv) $[T_3^-] = \frac{[OH^-] \text{ total ester}}{(1 + Z[OH^-])K_1}$

From iii) $[T_3^{--}] = \frac{[T_3^-][OH^-]}{K_2} = \frac{\text{total ester } [OH^-]^2}{(1 + Z[OH^-])K_1K_2}$

Hence rate $= \frac{r_1[OH^-]\text{total ester}}{(1 + Z[OH^-])K_1} + \frac{r_2 \text{ total ester } [OH^-]^2}{(1 + Z[OH^-])K_1K_2}$

$$= \frac{\text{total ester}}{1 + Z[OH^-]} (k_2[OH^-] + k_3[OH^-]^2).$$

where k_2 and k_3 are the rate constants for the bimolecular and termolecular paths of the reaction respectively.

The observed rate = k X total ester .

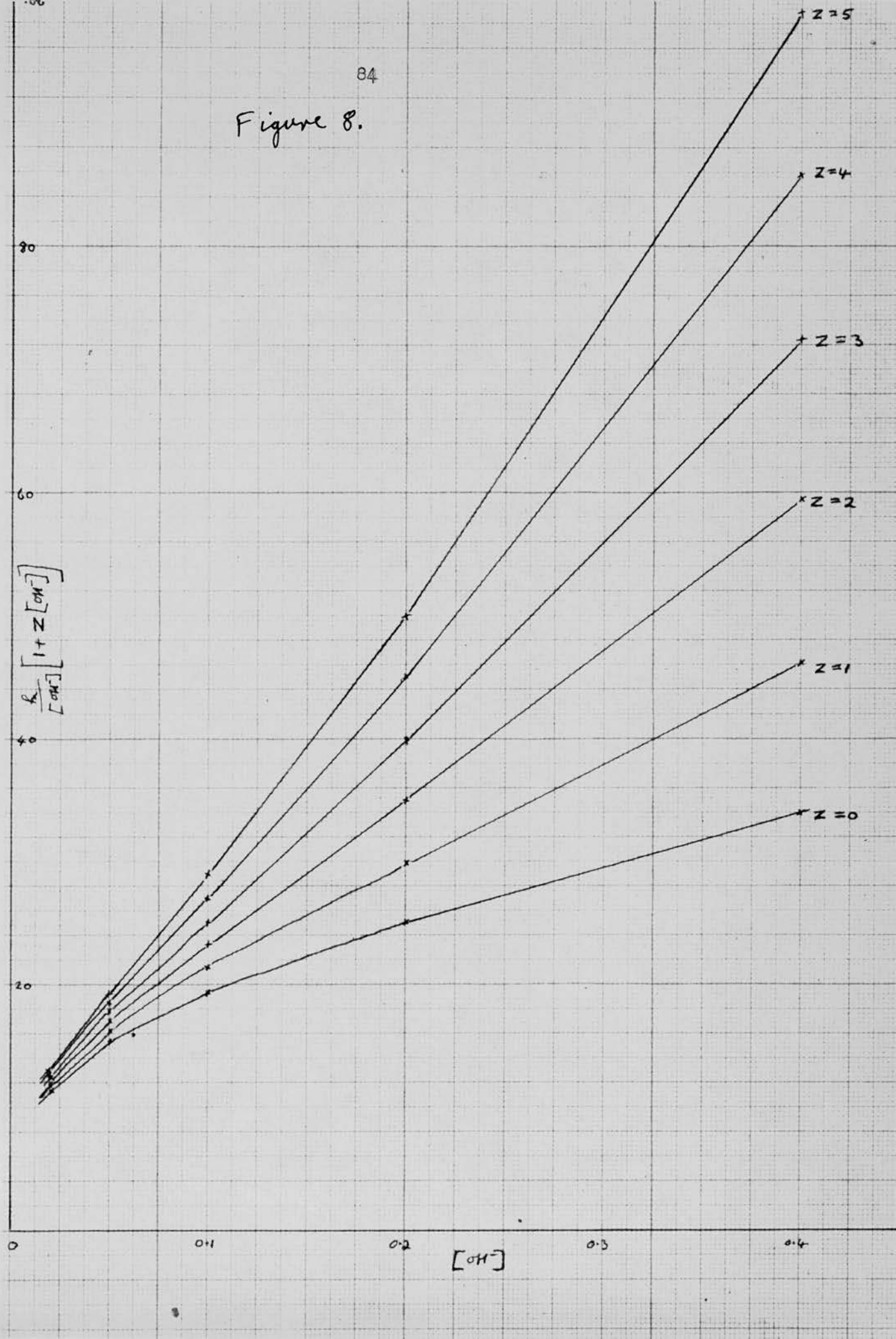
$$= \frac{\text{total ester}}{1 + Z[OH^-]} (k_2[OH^-] + k_3[OH^-]^2).$$

So that $$\frac{k}{[OH^-]} = \frac{k_2 + k_3[OH^-]}{1 + Z[OH^-]}.$$

According to this expression, $\frac{k}{[OH^-]}(1 + Z[OH^-])$ should be a linear function of $[OH^-]$ provided that the correct value of Z is chosen. In fact, when $\frac{k}{[OH^-]}(1 + Z[OH^-])$ is plotted against $[OH^-]$ for methyl 6-O-tosyl-α-D-glucoside for various values of Z, a family of curves is obtained, including a straight line when Z = 3(approximately) (Fig.8). For the condition Z=0 (i.e. assuming no appreciable concentration of sugar ion present) a marked deviation from linearity is observed and hence, the inclusion of the Z factor in the interpretation of the results for methyl 6-O-tosyl-α-D-glucoside is justified. Values of k_2 and k_3 can be obtained from the graph, but a more satisfactory way of calculating these is described later.

It can readily be shown that the quantity Z in the above expression is equal to $\frac{K_A}{K_W}$ where K_A is the first ionisation constant of the sugar ester and K_W is the ionic product of water. Michaelis[75] has obtained 1.97×10^{-14} for the ionisation constant of methyl α-D-glucoside at 18° (ionic strength ca.0.1M) and since K_W at 18° is 0.58×10^{-14} it follows that the value obtained above for Z is of the correct order of magnitude. This provides additional support for the Kinetic scheme postulated earlier.

Figure 8.

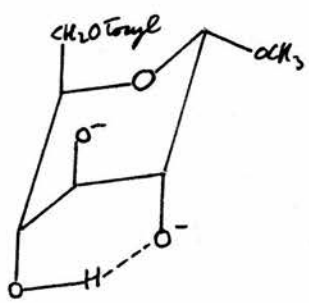


The experimental results for the galactose series require further consideration. The slight decrease in $\frac{k}{[OH^-]}$ with increasing concentration of sodium hydroxide is probably outside experimental error but the data does not allow the independent determination of k_3 and Z. If it is assumed that k_3 is negligibly small (i.e. that the reaction proceeds only via the mono-ion), then the data requires that for both anomers Z cannot be much greater than 0.5. This is unexpected, since the conductivity measurements described earlier suggest that the ionisation constants of methyl β -D-galactoside and methyl α -D-glucoside are similar. Therefore a more reasonable assumption seems to be that Z has approximately the same value for the galactosides as for the glucosides (i.e. Z = 2-3). The fact that $\frac{k}{[OH^-]}$ varies only slightly with increasing $[OH^-]$ in the case of the galactosides then indicates that $\frac{k_3}{k_2} \simeq Z$ (if these were exactly equal $\frac{k}{[OH^-]}$ would be independent of $[OH^-]$).

For the glucose series $\frac{k_3}{k_2} \simeq 10$ (see Table 17) i.e. much greater than Z. This leads to the conclusion that reaction via a doubly charged ion is much more important for the glucosides than for the galactosides. The position of 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol is not clear. The observed rate was essentially constant over the range of sodium hydroxide concentrations used and is probably quite close to the true value of k_2. Here again it is possible that $\frac{k_3}{k_2} \simeq Z$. The experimental data do not permit any derivation of k_3 to be made and no attempt was made to determine the pk of 1,5-anhydro-2-deoxy-D-glucitol. The alternative possibility that both k_3 and Z are negligible seems more likely in this case than for the galactosides since removal of the

electron-attracting oxygen functions at $C_{(1)}$ and $C_{(2)}$ would tend to depress the ionisation of the remaining hydroxyl groups.

A suggested explanation of the differences in kinetic behaviour is offered in terms of conformation and of hydrogen bonding between diaxial hydroxyl groups. Assuming that the transition state of the molecule is in the 1C form (see later discussion) then it is evident that the compounds in which the termolecular process plays an important part are those in which the hydroxyl groups at $C_{(2)}$ and $C_{(4)}$ bear a diaxial relationship to each other, whereas an axial-equatorial arrangement is to be found in methyl 6-O-tosyl-α and β-D-galactoside. 1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol, of course, has no hydroxyl group at $C_{(2)}$. The view is taken that under the influence of sodium hydroxide the molecule is ionised at $C_{(3)}$ and that in the case of those molecules having a diaxial arrangement of hydroxyl groups further ionisation at $C_{(2)}$ or $C_{(4)}$ is facilitated by stabilisation of the resulting anion by hydrogen bonding as shown below, thus favouring reaction via a di-ion.



Where the axial-equatorial relationship exists, the distances between the groups at $C_{(2)}$ and $C_{(4)}$ are too great for effective hydrogen bonding.

In support of this theory the unusual acidity of the 3,6-anhydro

derivatives of the methyl α and β -D-glucosides and of 1,5-anhydro-D-glucitol may be recalled. The differences between the infrared spectra of methyl 3,6-anhydro- α-D-glucopyranoside and methyl 3,6-anhydro- α-D-galactopyranoside are also in accordance with this view.

The graphical method of obtaining Z is rather imprecise since the "linearity" of the curves is not very sensitive to changes in Z. Hence only approximate values of the k_2 and k_3 can be obtained in this way. The method of least squares has therefore been used to obtain more precise values of the constants directly from the experimental data and the probable errors have been calculated (see appendix 1). For the glucose series (except for 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol) values for k_2 having 95% confidence limits of $\pm$ 10% were obtained, together with rather less accurate values of k_3 and Z. For reasons discussed earlier empirical values of Z had to be assumed in the case of the galactosides, and calculations were made using both Z = 2 and Z = 3. Satisfactory values for k_2 and somewhat less precise values for k_3 were obtained in this way. It may be noted that k_2 is insensitive to the value assumed for Z but that k_3 is greatly affected. In the case of 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol k_2 was obtained simply by averaging the observed values of $\frac{k}{[OH^-]}$.

The values of k_2, k_3 and Z obtained by the method of least squares are shown in Table 17.

TABLE 17.

Compound	$10^4 k_2$	$10^2 k_3$	Z
Methyl 6-O-tosyl-α-D-glucoside.	10.04 ± 0.94†	1.34 ± 0.24†	2.33 ± 0.68†
Methyl 6-O-tosyl-β-D-glucoside.	6.77 ± 0.19	0.573± 0.03	2.05 ± 0.35
1,5-Anhydro-6-O-tosyl-D-glucitol.	102.2 ± 7.3	8.66 ± 1.52	3.3 ± 1.53
Methyl 6-O-tosyl-α-D-galactoside.	78.8 ± 0.8	1.10 ± 0.15	2 *
	79.3 ± 0.8	1.77 ± 0.17	3 *
Methyl 6-O-tosyl-β-D-galactoside.	55.1 ± 0.1	0.92 ± 0.03	2 *
	55.2 ± 0.2	1.43 ± 0.04	3 *
1,5-Anhydro-2-deoxy-6-O-tosyl-D-glucitol.	93.3 @	-	-

* The values of Z were assumed in order to calculate k_2 and k_3. (See appendix).

@ Mean value of $\frac{k^G}{[OH^-]}$ for all concentrations of sodium hydroxide.

† 95% confidence limits.

2) Conformational aspects of the reaction.

The primary object of the measurement of the rate constants described in this work was an attempt to determine in a quantitative manner the nature and magnitude of the forces which are responsible for the preferred conformation of any particular compound. In addition to the well established effects of non-bonded interactions due to the proximity of groups in certain structures, a number of other factors are thought to be of some importance in the sugar series. Of these, dipole interactions and hydrogen bonding, may have a considerable influence in determining conformations. The results of the kinetic measurements described in the present work are discussed in these terms in the following pages. On the basis of this work no absolute values of the magnitudes of non-bonded interactions can be assigned but some useful qualitative deductions are made.

The observed first-order rate constants have no direct significance so far as conformation is concerned since the reaction proceeds by two distinct paths. However, a comparison of the derived second-order constants is of some interest. According to the proposed mechanism, k_2 is a composite quantity, being the product of the equilibrium constant for the ionisation of the tosyl ester at $C_{(3)}$ and a first-order rate constant. Changes in structure can affect k_2 through either of these quantities. This complicates the interpretation of the results and a consideration of each of these factors is necessary.

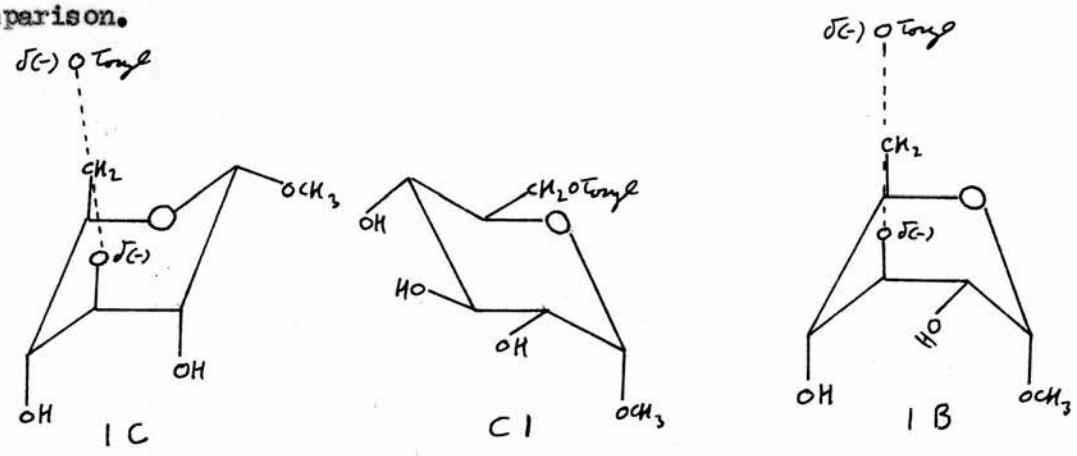
The removal of electron-attracting oxygen functions would be expected to decrease the ionisation of the hydroxyl group at $C_{(3)}$ and would therefore

retard the reaction. Since electronic effects are not readily transmitted in saturated systems, it is unlikely that this effect will be great unless one of the adjacent hydroxyl groups is involved. The operation of this factor is clearly seen by comparing k_2 for the tosylates of 1,5-anhydro-D-glucitol and its 2-deoxy-derivative. Because of the smaller increase of non-bonded interactions on conversion into the transition state, the latter compound would be expected to react faster, but, if fact, the values of k_2 for the two compounds are similar. The removal of the hydroxyl group at $C_{(2)}$ evidently slows the reaction and neutralises the steric influence. Removal of the more remote glycosidic methoxyl group however increases the rate by a factor of at least ten, as is shown by comparing k_2 for the tosylates of methyl α and β-D-glucoside and that of 1,5-anhydro-D-glucitol.

Inductive effects on the ease of displacement of the tosyl group at $C_{(6)}$ must also be considered. It is likely that in the nucleophilic displacement reactions of sulphonyl esters the carbon atom develops a + ve charge[76]. The removal of electron-attracting substituents would therefore by expected to facilitate replacement of the tosyloxy group. The greater rate of reaction for 1,5-anhydro-6-O-tosyl-D-glucitol over either of the glucoside esters may be explained in this way but it is unlikely that this factor is entirely responsible, because of the remoteness of the glycosidic methoxyl group from $C_{(6)}$. Steric effects may be involved as discussed later. A further study of 2-deoxy glycoside tosylates would be of possible interest in the determination of electronic effects.

Since the ionisation constants of the $C_{(3)}$ - OH in the 6-O-tosyl

glucosides and galactosides (and probably in 1,5-anhydro-6-O-tosyl-D-glucitol) can be assumed to be similar, the differences in k_2 for these compounds must be due mainly to steric factors. A fundamental question which must be asked before the effect of configuration on k_2 can be discussed in conformational terms is the conformation of the transtion state for the reaction. Since the reaction requires that the carbon atom $C_{(6)}$ and the oxygen atom at $C_{(3)}$ should be close together, skew conformations are excluded and only one boat form and one chair form need be considered. These are shown below for the transition state from the α-glucoside; The most probable conformation of the original tosylate is also shown for comparison.



For each compound in the transition state a boat or a chair form is possible and the fact that the transition state may not have the same conformations for all of the compounds in the present series must be considered. Foster, Overend and Vaughan[67] have suggested, on the basis of arbitrary "interaction units", that some 3,6-anhydroglycopyranosides have chair conformations while others exist as boats; there is however, no direct experimental

evidence in support of these suggestions and in particular the possible importance of dipole interactions is neglected.

The present results do not permit entirely unambiguous assignments of the conformations of the transition states to be made. The subsequent discussion is based on the assumption that chair conformations are involved; there is, in fact, circumstantial evidence from three sources in favour of this view.

First, methyl 3,6-anhydro- α-D-glucoside and its β-anomer both show very similar, abnormally high ionisation in sodium hydroxide solution. As suggested earlier, this is best explained in terms of facilitation of the ionisation by hydrogen bonding - an effect which is only possible in the chair conformations. It therefore seems likely that the transition states for the formation of these compounds also have chair conformations.

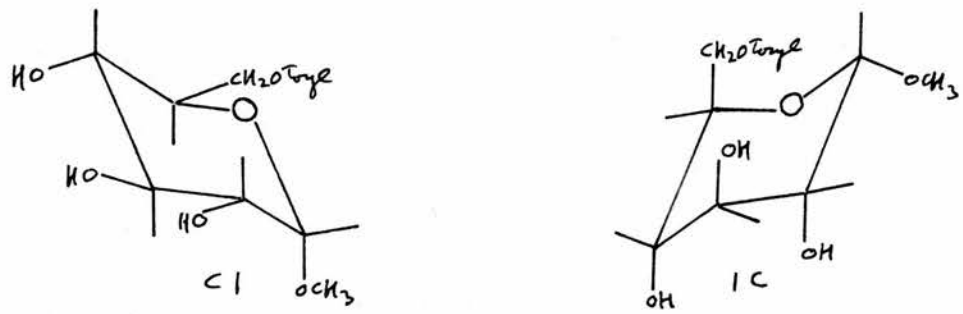
Secondly, the work of Reeves has shown that 1,6-anhydro-D-glucose adopts the "all-axial" chair conformation in preference to a boat conformation[65]. This suggests that in structures in which it cannot be "skewed", the boat conformation has very low stability.

Thirdly, the ratio $\frac{k_2\ (\alpha)}{k_2\ (\beta)}$ is similar for both glucoside and galactosides, This is readily explicable if the transition states in all four cases have chair conformations. If appreciable amounts of boat conformations were present in some of the compounds, identity of the above ratios would be unlikely, since boat conformations are less favoured for the glucoside transition states because the 4-OH group is at a "flagpole" position (in the case of the α-glucoside, for example,

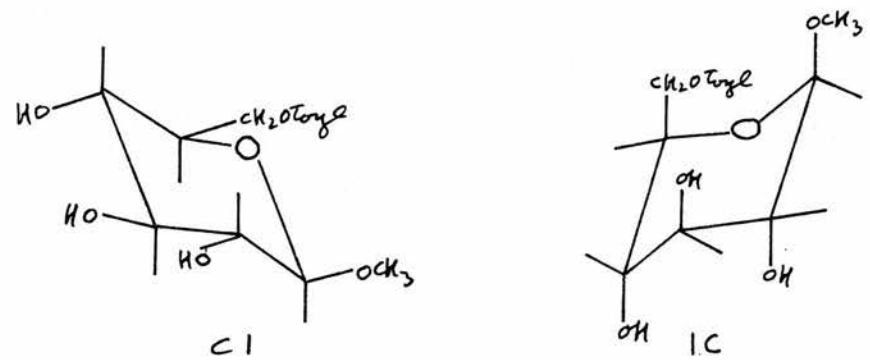
the boat transition state would involve prohibitive interactions between oxygen functions at both "flagpole" positions).

However, the above evidence is by no means conclusive and definite evidence on the conformations of the 3,6-anhydro glucosides would be of great interest, particularly for methyl 3,6-anhydro-β-D-glucoside and galactoside, to which Foster et al[67] have assigned boat conformations. Nuclear magnetic resonance measurements may provide the required evidence.

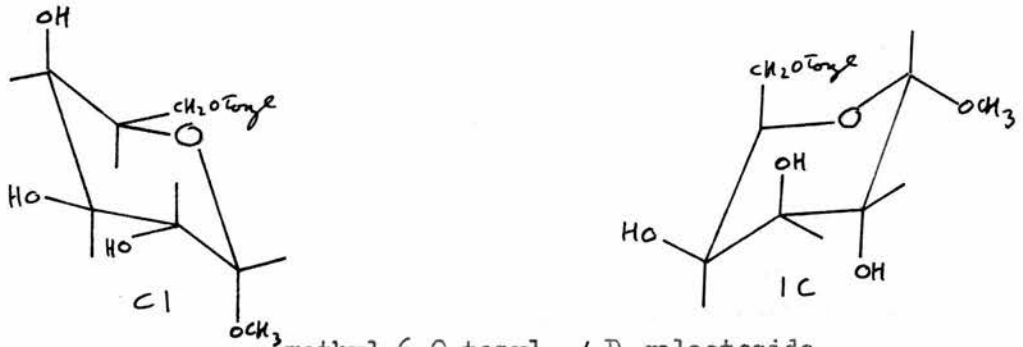
If it is assumed that the inductive effects discussed earlier are constant for the various glycoside tosylates, a comparison of the values of k_2 for these compounds is indicative of the operation of conformational factors.



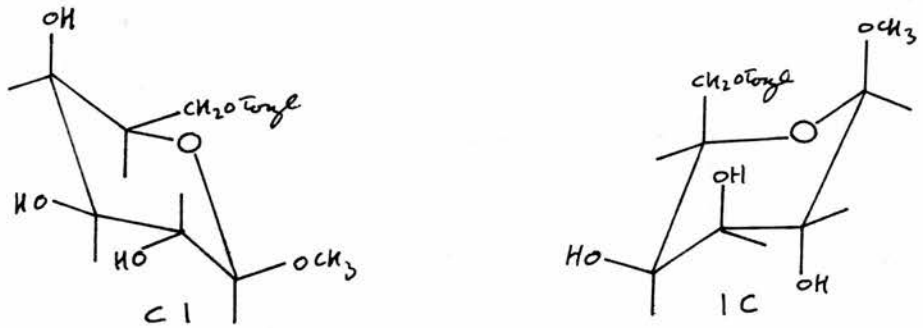
methyl 6-O-tosyl-α-D-glucoside



methyl 6-O-tosyl-β-D-glucoside



methyl 6-O-tosyl-α-D-galactoside



methyl 6-O-tosyl-β-D-galactoside

For the series of compounds illustrated above,

$$\frac{k_2\ \alpha\ \text{gal.}}{k_2\ \alpha\ \text{gluc.}} \rightleftharpoons \frac{k_2\ \beta\ \text{gal.}}{k_2\ \beta\ \text{gluc.}} \rightleftharpoons 8.$$

this result is expected on conformational grounds since in the 1C conformations the 4-OH group in the galactosides assumes an equatorial position and consequently the non-bonded interactions are less than in the glucosides. Similarly the fact that $\frac{k_2\ \alpha\ \text{glu.}}{k_2\ \beta\ \text{glu.}} \rightleftharpoons \frac{k_2\ \alpha\ \text{gal.}}{k_2\ \beta\ \text{gal.}} \rightleftharpoons 1.5$ may be explained in terms of the equatorial position of the methoxyl group in the 1C conformation of the alpha anomer.

However, it is remarkable that the differences between the rates of reaction of the α and β compounds are considerably smaller than the differences between the glucosides and galactosides. On the grounds of steric repulsions alone the opposite would have been expected since in the 1C conformation an axial methoxyl group at $C_{(1)}$ encounters

repulsions from both $C_{(6)}$ and $O_{(3)}$, whilst an axial OH at $C_{(4)}$ only encounters repulsions from $O_{(2)}$. Two possible explanations of this discrepancy, namely "dipole interactions" and "passing interactions", will be considered in turn.

In the equilibrium mixture of methyl α and β -D-glucopyranosides and similarly for other anomeric mixtures, the α form predominates; furthermore, the α -anomers are less readily hydrolysed. This is unexpected since in the C1 conformations the glycosidic methoxyl group of the α anomer is axial. Edward[30] has explained the effect in terms of "electrostatic repulsive forces"; i.e. dipole interactions between the dipole due to the ring oxygen and the dipole of the glycosidic methoxyl group. It was considered that these favour the oxygen of the glycosidic group being in the axial rather than the equatorial orientation. Hordvik[77] has critised this view; he points out that since there is free rotation about the $C_{(1)}$-O bond, the dipole interactions can vary and "may be as considerable for an axial as for an equatorial group".

Calculations based on molecular models (Inglis and Schwarz, private communication) show that both for an axial and an equatorial methoxyl group either attractions or repulsions may arise, depending on the rotational orientation of the methyl group. In these calculations the dipoles were assumed to be point dipoles located at the centres of the oxygen atoms and directed along the bisectors of the C-O-C angles. The results suggest that in the case of an equatorial methoxyl, dipole attractions occur only for sterically unfavoured orientations of the

methyl group; for sterically ~~unfavoured~~ favoured orientations the interaction is either a repulsion or zero. For an axial methoxyl, however, there are sterically favoured orientations for which the dipole interaction is an attraction. The calculations must be regarded as tentative, since the magnitudes and even the signs of the calculated interactions change if the point dipole is moved along its axis. Furthermore, the assumption of point dipoles is certainly an oversimplication. Nevertheless the results suggest that it is not implausible to ascribe the greater stability of axial orientations in simple glycosides and related compounds to attractive dipole-interactions, which favour the axial orientation; there may also be some destabilisation of the equatorial orientation. Thus the dipole-attractions act in opposition to purely steric repulsions and the unexpectedly small value of the ratio $\frac{k_2\,\alpha}{k_2\,\beta}$ for the glucosides and galactosides becomes explicable.

Dipole effects may also be responsible for the large difference in the rates of cyclisation of 1,5-anhydro-6-O-tosyl-D-glucitol and methyl 6-O-tosyl-α-D-glucoside ($\frac{k_2\ 1,5\ \text{anhydro.}}{k_2\ \alpha\text{-glu.}} \simeq 10$). As noted earlier this difference is unlikely to be entirely due to inductive effects. However, in the conversion of the C1 conformation of methyl 6-O-tosyl-α-D-glucoside into the 1C form, the orientation of the glycosidic methoxyl group changes from axial to equatorial. This change is favoured on purely steric grounds but is opposed by dipole factors; as mentioned above the position of the anomerisation equilibrium for the methyl α-D-glucopyranosides shows that the dipole factor is more important. Hence 1,5 anhydro-6-O-tosyl-D-glucitol

in which the methoxyl group is absent would be expected to cyclise more quickly than the α-glucoside derivative.

To confirm the importance of dipole interactions it would be of interest to examine the rates of cyclisation of the above compounds in a solvent such as aqueous dioxan, in which the effect of dipole-interactions would be enhanced, both because of the lower dielectric constant and because of decreased solvation of the dipoles.

The conversion of a molecule from one chair conformation to the other must, if cis substituents are present, involve eclipsing of these groups at some time during the interconversion. Newth[4] has suggested that such "passing interactions" may account for differences in reactivity between stereoisomers in certain reactions giving 3 - membered oxide rings. Since the methyl 6-O-tosyl-α-D-glycosides of galactose and glucose each have cis substituents at $C_{(1)}$ and $C_{(2)}$ it might be thought that the operation of "passing interactions" could account for the relatively small difference between the rates of cyclisation of the corresponding α and β anomers in each series. However, there are a number of objections to this view.

The first is that "passing interactions" would retard the cyclisation of the galactosides relative to that of the glucosides since in the former the hydroxyl groups at $C_{(3)}$ and $C_{(4)}$ and the tosyloxymethylene group at $C_{(5)}$ are all in this cis position. When this second effect (which is probably greater than the interactions between substituents at $C_{(1)}$ and $C_{(2)}$, as two pairs of cis - substituents are involved) is taken into account, it is clear that "passing interactions" cannot be responsible for the low ratio of $\frac{k_2 \alpha}{k_2 \beta}$.

A second objection is a kinetic one. "Passing-interactions" are in fact energy barriers to the interconversion of alternative conformations and if they are to affect the rates of reaction, this interconversion must be slow, compared with the other steps of the reaction. In fact, the kinetic scheme discussed earlier was derived on the implicit assumption (usually made in such circumstances),[31,32,78] that the interconversion of conformations is fast compared with other steps in the reaction. If this is not the case and "passing-interactions" are kinetically significant, the rate equation derived earlier is not adequate. A rigorous treatment which takes account of "passing-interactions" does not seem feasible, but an approximate treatment using the stationary state assumption and considering only reaction via a singly charged ion leads to the expression,

$$\frac{k}{[OH^-]} = \frac{k_2 + a[OH^-]}{1 + b[OH^-] + c[OH^-]^2}$$ where k_2 is the second-order rate constant in the absence of significant "passing-interactions" and a,b and c are constants (Inglis and Schwarz, private communication).

According to this expression the extrapolated value of $\frac{k}{[OH^-]}$ at zero hydroxide concentration is equal to k_2 and is therefore independent of passing-interactions. A similar conclusion can be reached intuitively since at infinitesimal hydroxide concentrations conformational interconversion can no longer remain a rate determining step. It follows therefore that although "passing-interactions" might influence the observed rate constant at finite hydroxide concentrations, they cannot account for the difference in $k_2 = \lim_{[OH^-] \to \text{zero}} \frac{k}{[OH^-]}$ discussed above.

Experimental confirmation of the conclusion that passing-interactions cannot be responsible for the low value of the ratio $\frac{k_2\,\alpha}{k_2\,\beta}$ for the glucoside could possibly be obtained by examining the corresponding 2-deoxy compounds.

It may be noted in passing that this reaction scheme does not explain the increase of $\frac{k}{[OH^-]}$ with $[OH^-]$ which was found in the present work, since it can be shown that b is greater than $\frac{a}{k_2}$. The assumption made earlier that part of the reaction occurs via a doubly charged ion is therefore still necessary.

EXPERIMENTAL

Apparatus for kinetic measurements.

All spectroscopic measurements were made using a Unicam SP. 500 spectrophotometer fitted with a Unicam SP. 570 constant temperature cell housing. Water from a bath maintained constant at 19.85° ± 0.03° by means of a commercially available Shandon Circotherm thermostat was circulated through the cell housing using a Stuart Turner No. 10 pump which was situated in the return stream from the cell holder; a copper cooling coil immersed in the bath was necessary to overcome the heating effect of the thermostat motor. It was found that the proximity of the hydrogen lamp to the cell housing caused a rise in temperature of the cells of 0.19° above the temperature of the bath; in order that the system should be in equilibrium, the hydrogen lamp was kept switched on permanently and in this way a cell temperature of 20.04 ± was achieved. The measurement of cell temperature

was made by means of an N.P.L. calibrated thermometer immersed in a cell filled with glycerol and seated in the cell housing. A hole was drilled through the loose cover of the cell housing for this purpose. It was necessary to remcve the thermometer for the duration of the kinetic runs but a frequent check was made of the temperature. The apparatus was assembled for use in a room the temperature of which was held constant at 21 ± 1° by means of a thermostat, as large variation in the ambient temperature were found to have an adverse effect on the temperature control of the cell.

Stability of 6-tosyl-esters in three molar sodium chloride solution.

Approximately 0.001M solutions were prepared by dissolving a weighed quantity of the appropriate tosyl ester in distilled water (50 ml.). Analytical reagent quality sodium chloride was then added and the solution diluted accurately in a graduated flask so that the final solution was 3M with respect to sodium chloride. The solutions were maintained at 21 ± 1° and the optical density at 265 mμ measured in 1 cm. cells at intervals of several days over a considerable period. Because of its sparing solubility, measurements on methyl 6-O-tosyl-D-galactoside were made at 240 mμ using 0.0001M solutions of tosyl ester in a 4 cm. cell in order to obtain a suitable range of optical density (see page 106). All measurements were made against a cell containing a 0.001M solution of sodium tosylate in distilled water. The absorption of the blank was checked each time by measuring against air and small changes in this value were allowed for in the subsequent measurement of the absorption of the solution under test.

The experimental results are shown in Tables 9 and 10.

T A B L E 9.

The stability of 1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol in water at 21°.

Time in days.	0	10	21	78
Optical density (E) of 0.001M solution 1,5-anhydro-2-deoxy -6-O-tosyl-D-glucitol, measured at .265 mμ against 0.001M sodium tosylate.	0.452	0.395	0.312	0.091

Preparation of solutions for kinetic measurements.

For measurements at 265 mμ, 0.002M solutions of the tosyl compound were prepared by dissolving the required quantity of material (usually sufficient to make 100 ml. of 0.002M solution) in 80 ml. of deionised water in a standard flask. The amount of analytical reagent quality sodium chloride required to give a 3M solution was then added and dissolved. The solution was made up to the mark using deionised water; care was taken to mix thoroughly before making up the volume accurately as considerable contraction in volume occurs on mixing. For the less soluble methyl 6-O-tosyl-α-D-galactoside, 0.0002M solutions were prepared similarly.

Sodium hydroxide solutions of the required strength were obtained by diluting a suitable aliquot of 1.0N or 0.1N sodium hydroxide solution made from B.D.H. concentrated volumetric solutions. The B.D.H. solutions were shown to be essentially free from carbonate

TABLE 10.

The stability of the 6-O-tosyl compound in 3M sodium chloride 21 ± 1°.

Optical density (E), measured at 265 mμ against 0.001M sodium tosylate,

of 0.001M solutions of:-

Time in days	methyl 6-O-tosyl-α-D-glucoside	methyl 6-O-tosyl-β-D-glucoside	methyl 6-O-tosyl-β-D-galactoside	methyl 6-O-tosyl-α-D-galactoside	1,5-anhydro-6-O-tosyl-D-glucitol	1,5-anhydro-2-deoxy-6-O-tosyl-D-glucitol
0	0.521	0.541	0.516	* 1.006	0.533	-
1	-	-	-	-	-	0.503
7	0.492	0.513	0.420	0.987	0.500	0.411
14	0.470	0.501	0.364	-	0.486	0.346
16	-	-	-	0.069	-	-
21	0.456	0.500	0.327	0.960	0.480	0.297
27	-	-	-	0.948	-	-
28	0.441	0.485	0.294	-	0.465	0.256
35	0.422	0.473	0.265	0.927	0.449	0.212
42	0.398	0.460	0.231	-	0.434	0.175
49	0.393	0.459	0.215	-	0.424	0.154
58	0.386	0.459	0.190	-	0.420	0.130
63	0.379	0.449	0.172	-	0.411	0.111

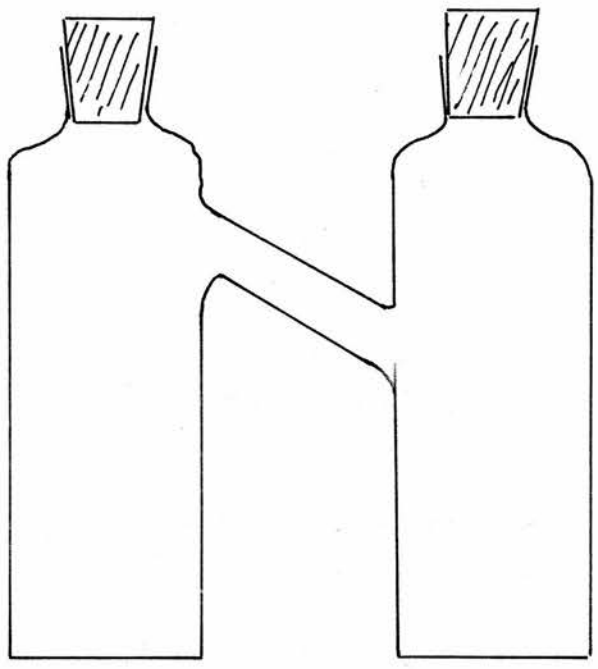
* 0.0001M solution measured in 4 cm. cell at 240 mμ.

by titration with sulphuric acid using methyl orange and phenolphthalein indicators. Before diluting the final solution to a standard volume sufficient sodium chloride of analytical reagent quality was added to bring the total ionic strength of the solution to 3M.

By mixing equal volumes of the prepared solutions the required concentrations of reactants was obtained and changes in volume due to dilution effects were avoided.

Kinetic Procedure

Figure 9.



The reaction vessel.

The prepared solution of tosyl ester (10 ml.) was pipetted into one arm of the reaction vessel (Fig. 9) and the glass stopper inserted. The air in the apparatus was displaced by means of a stream of nitrogen, and sodium hydroxide solution (10 ml.) transferred to the other arm of the vessel by means of a pipette. A second stopper was loosely inserted and the vessel immersed in a water bath at 35-40° for 5 minutes. This precaution was taken to expel dissolved air which otherwise gives rise to air bubbles on the cell walls during the reaction. The solution of sodium tosylate against which measurements were being made (see below) was treated similarly. The reaction vessel was then immersed, up to the lower level of the stoppers, in the thermostat bath and allowed to come to equilibrium with it. The slight difference between bath temperature and the actual temperature in the cell is not sufficient to affect the reaction rate.

In the meantime two 1 cm. silica cells were placed in position in the cell housing; one of these was filled with 0.001M sodium tosylate solution to serve as a blank. When cells and reaction vessel had come to thermal equilibrium with their surroundings (usually about 30 minutes was allowed for this), the reaction vessel was removed from the bath and the solutions in the two arms mixed as quickly as possible by inverting the cell several times; the time commencement of mixing was noted. Using a pipette, specially constructed to deliver the required volume, a portion of the solution was transferred to the cell in which the reaction was to be followed. These operations could, if necessary, be done sufficiently rapidly to allow the first reading to be taken within one minute of mixing the solutions. It was advisable to "zero" the dark current and blank cell of the spectrophotometer immediately

prior to mixing the solutions as this enabled the first reading to be taken quickly. As a check on instrument performance during the experiment the one hundred per cent transmission was checked before commencing each run and the optical density of the blank solution was measured against air before and after each experiment.

After starting the experiment it was found inadvisable and unnecessary to touch the cells. The blank was "zeroed" by means of the slit width control, and in no circumstances was the wavelength setting, which had been preset to the required value (265 mμ in most cases) adjusted. In cases where readings were being taken at intervals greater than one minute the blank solution was returned to the light beam and the zero checked after each reading. For shorter time intervals this was not possible and occasional checks only were made. Readings were taken at constant time intervals over at least three and preferably four half-lives; after approximately two half-lives when differences in optical density became small, the time interval was usually doubled and the other points obtained by graphical interpolation. A final reading was usually taken after some twelve half-lives when the reading had become constant.

Kinetic measurements.

The first-order rate constant for each compound was determined at five different concentrations of sodium hydroxide in the range 0.02 - 0.4N using at least a twentyfold excess of alkali. At least two, usually three runs were carried out at each concentration. Usually the change in optical density was observed at 265 mμ using 1 cm silica

cells. In this way a total change in optical density of approximately 0.5 units was obtained. In order that the readings could be taken from the more accurately readable part of the instrument scale, measurements were made using an equivalent concentration of sodium tosylate as a blank. In theory this should lead to a final value of zero for the optical density but this was seldom found to be the case. Minor deviations usually occurred and the experimental end value was always used for calculations. As stated elsewhere, there was sometimes a slight rise in absorption at the end of the reaction and in these cases the minimum observed end value was used.

The sparing solubility of methyl 6-O-tosyl-α-D-galactoside prevented the preparation of 0.001M solutions in 3M sodium chloride and for measurements on this compound 0.0001M solutions were used. The reactions were carried out in 4 cm. silica cells and the optical densities measured at 240 mμ under which conditions a suitable change in optical density was observed. Although the slope of the absorption curve for the 6-tosyl ester is very great at this wavelength (see p. 66, Fig. 7) the reproducibility of the results was in no way affected.

For methyl 6-O-tosyl-α-D-glucoside additional measurements were made in which the concentration of the sugar ester was varied within the limits 0.005 - 0.0005M, the sodium hydroxide concentration being kept constant at 0.4M. In order to obtain a convenient range of optical density, measurements at the higher concentration of tosyl ester were made using 2 mm. cells at a wavelength of 265 mμ. For the lower concentrations 1 cm. cells were used and the measurements made at a

wavelength of 240 mμ.

In all these experiments the toal ionic strength was kept constant at 3M by the addition of sodium chloride.

Rate constants were obtained graphically using the Guggenheim method and the end value method in each case. The reproducibility of the results was generally within less than 5%; obviously inaccurate runs were rejected. The average degree of agreement between the Guggenheim method and the direct method when compared for the same set of experimental data was within 2%.

Typical sets of experimental data Tables 18 and 19 and the plots obtained from them are shown on pages 108-111. Column 2 of Table 18 illustrates the rise in the value of the absorption at the end of the reaction which occurred in some cases and which has been referred to previously. The rate constants are tabulated on pages 73-78.

TABLE 18

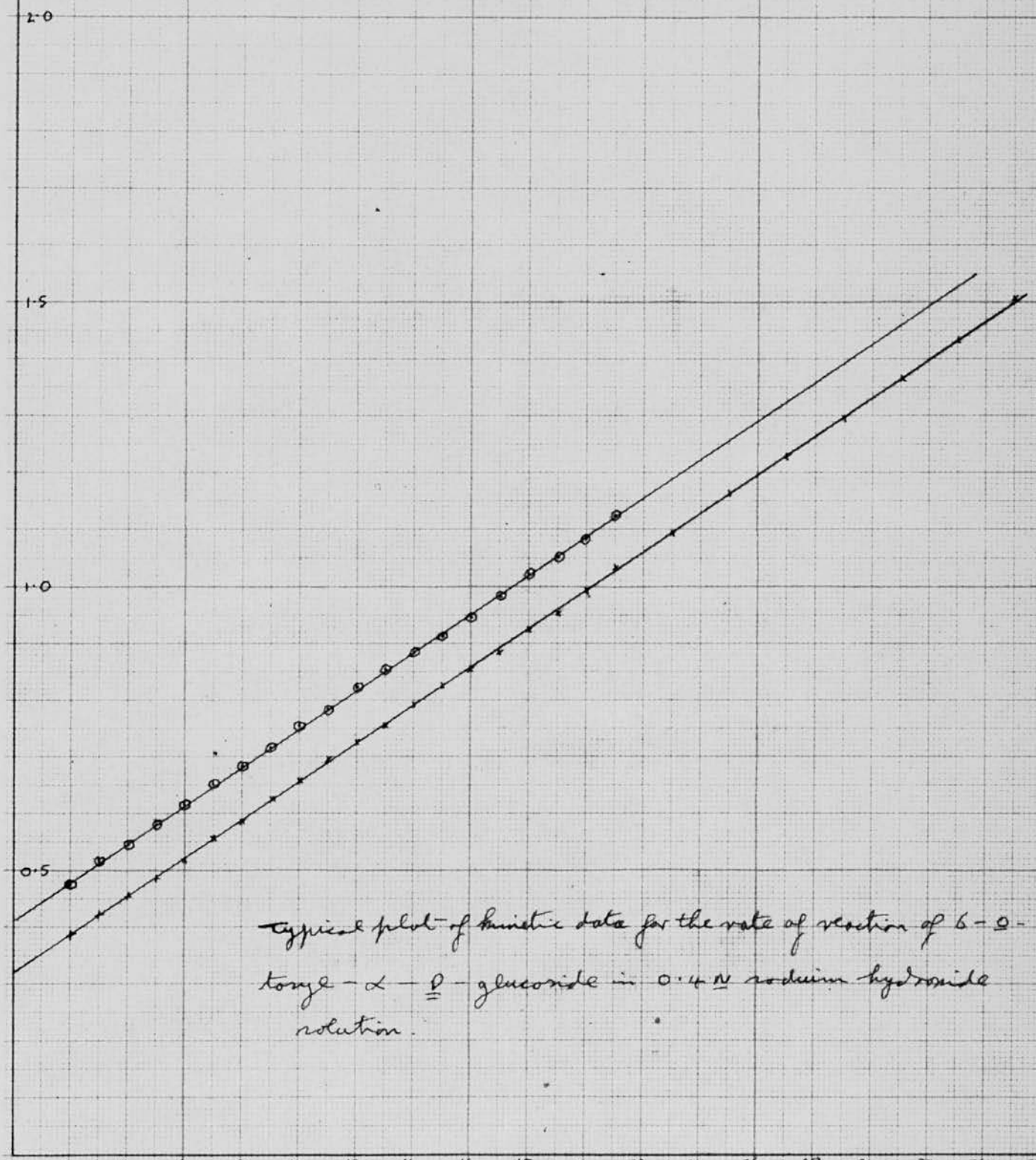
Typical experimental data

for the reaction of methyl 6-O-tosyl-α-D-glucoside in 0.4N sodium hydroxide at 20.04 ± 0.03°

Time in mins.	E_t	$\log \frac{1}{E_t - E_\infty}$ *	$\log \frac{1}{E_t - E_{(t+r)}}$ ⊙
0	-	-	-
2	0.482	0.382	0.4757
3	0.448	0.4191	0.5139
4	0.420	0.4523	0.5466
5	0.393	0.4867	0.5817
6	0.370	0.5185	0.6126
7	0.345	0.5560	0.6507
8	0.325	0.5884	0.6829
9	0.306	0.6216	0.7156
10	0.287	0.6576	0.7520
11	0.270	0.6925	0.7879
12	0.255	0.7258	0.8211
13	0.242	0.7569	0.8507
14	0.229	0.7905	0.8827
15	0.217	0.8239	0.9137
16	0.206	0.8569	0.9469
17	0.1955	0.8894	0.9829
18	0.1850	0.9282	1.0224
19	0.177	0.9586	1.0508
20	0.168	0.9956	1.0864
21	0.160	1.0314	1.1222
23	0.1475	1.0941	
25	0.136	1.1612	
27	0.126	1.2292	
29	0.1175	1.2967	
31	0.110	1.3666	
33	0.104	1.4319	
35	0.098	1.5087	
75	0.068		
90	0.067		
120	0.067		
1500	0.082		

* E_∞ = 0.067
⊙ r = 23 mins.

X Plot of data in column 3 of table 18.
O Plot of Guggenheim data in column 4 of table 18.



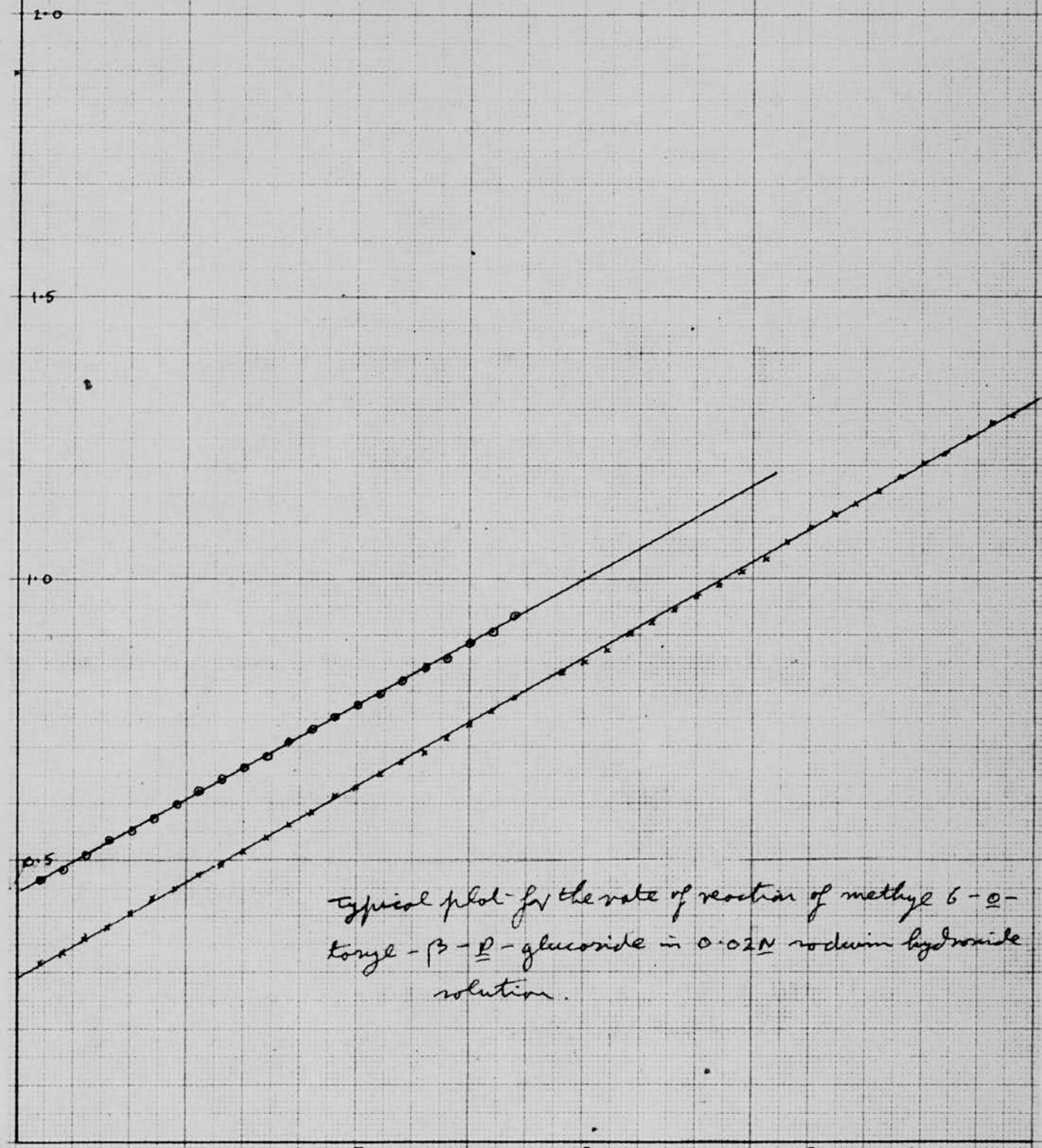
Typical plot of kinetic data for the rate of reaction of 6-O-tosyl-α-D-glucoside in 0·4 N sodium hydroxide solution.

TABLE 19

Typical experimental data for the reaction of methyl 6-O-tosyl-β-D-glucoside with 0.02N sodium hydroxide at 20.04 ± 0.03°

Time in hours	E_t	$\log \frac{1}{E_t - E_\infty}$	$\log \frac{1}{E_t - E_{(t+r)}}$
0	-	-	-
1	0.523	0.3179	0.4660
2	0.500	0.3391	0.4874
3	0.477	0.3615	0.5087
4	0.455	0.3840	0.5308
5	0.433	0.4079	0.5551
6	0.413	0.4306	0.5783
7	0.395	0.4523	0.6003
8	0.378	0.4737	0.6216
9	0.361	0.4962	0.6440
10	0.344	0.5199	0.6656
11	0.329	0.5421	0.6861
12	0.314	0.5654	0.7100
13	0.302	0.5850	0.7305
14	0.287	0.6143	0.7569
15	0.276	0.6308	0.7746
16	0.264	0.6537	0.7959
17	0.253	0.6757	0.8196
18	0.243	0.6968	0.8386
19	0.233	0.7190	0.8601
20	0.223	0.7423	0.8827
21	0.213	0.7670	0.9101
22	0.204	0.7905	0.9356
24	0.189	0.8327	
25	0.181	0.8569	
26	0.175	0.8761	
27	0.167	0.9031	
28	0.161	0.9245	
29	0.155	0.9469	
30	0.149	0.9706	
31	0.144	0.9913	
32	0.139	1.0133	
33	0.134	1.0362	
34	0.128	1.0656	
35	0.123	1.0917	
36	0.119	1.1137	
37	0.116	1.1306	
38	0.112	1.1561	
39	0.108	1.1804	
40	0.104	1.2076	
41	0.102	1.2219	
42	0.098	1.2519	
43	0.095	1.2758	
44	0.093	1.2925	
	0.042	-	

× Plot of data in column 3 of table 19.
⊙ Plot of Guggenheim data in column 4 of table 19.



Typical plot for the rate of reaction of methyl 6-O-tosyl-β-L-glucoside in 0·02N sodium hydroxide solution.

Appendix

Derivation of the rate constants k_2 and k_3 by the method of least squares.

The rate equation for the reaction between sodium hydroxide and the 6-O-tosyl glycosides may be written in the form

$$\frac{k}{[OH^-]} 1 + Z\,[OH^-] = k_2 + k_3[OH^-]$$

If values of Z, taken as a whole number in the range 0-10, are substituted in the equation the resulting plot of $\frac{k}{[OH^-]} 1 + Z[OH^-]$ against $[OH^-]$ provides a family of curves which includes a straight line for one of the values of Z. The slope of this line represents k_3 and the intercept is k_2. The method is rather subjective and it was desirable to obtain more reliable values for the rate constants. For this purpose a statistical procedure based on the method of least squares was adopted. The procedure was suggested by Dr. D.N. Lawley (Department of Statistics, University of Edinburgh) to whom the author wishes to express his thanks.

Considering the rate equation in the form

$$\frac{k}{[OH^-]} = k_2 + k_3[OH^-] - Z\,k \qquad \text{(See footnote)}$$

If E is the error in any one set of experimental values and denotes the summation over all the observations (thus in $[OH^-]$ a value of $[OH^-]$ is counted twice if duplicate determinations were carried out on this concentration),

Footnote.

An assumption implicit in this treatment is that $\frac{k}{[OH^-]}$, $[OH^-]$ and k are all independent variables. This is not strictly correct but the results obtained on application of the method justify the assumption.

then i) $E = k_2 + k_3[OH^-] - Z\,k - \frac{k}{[OH^-]}$.

and ii) $E^2 = (k_2 + k_3[OH^-] - Z\,k - \frac{k}{[OH^-]})^2$.

The best values of the parameters k_2, k_3 and Z are chosen such that $\sum E^2$ is a minimum. Mathematically the Least Square "normal" equations for estimating the parameters may then be represented as follows:-

$$\frac{d\sum E^2}{dk_2} = 0. \qquad \frac{d\sum E^2}{dk_3} = 0. \qquad \frac{d\sum E^2}{dZ} = 0.$$

These result in the following equations which may be solved for k_2, k_3 and Z.

iii) $Nk_2 + k_3\sum[OH^-] - Z\sum k = \sum\frac{k}{[OH^-]}$

iv) $k_2\sum[OH^-] + k_3\sum[OH^-]^2 - Z\sum k[OH^-] = \sum k.$

v) $k_2\sum k + k_3\sum k\,[OH^-] - Z\sum k^2 = \sum k^2.$

where N is the total number of observations made.

The values of k_2, k_3 and Z obtained in this way may be substituted in equation i) and the value of E calculated for each of the N observations using the known value of $[OH^-]$ and k. A random variation in E provides some support for the validity of the method.

In order to determine the accuracy of the parameters obtained by this method, the standard errors were calculated as follows. The standard error (S) in any observation is defined as $\sqrt{\text{variance}}$.

The residual variance of $\frac{k}{[OH^-]}$ is given by the expression $S^2 = \frac{\sum E^2}{N-P}$ ....(vi), where N-P is the number of degrees of freedom of the system, N being the total number of observations as previously defined and P the number of parameters to be determined (in this instance P=3).

The value of $\sum E^2$ can be obtained from the equation

$$\Sigma E^2 = \Sigma\left(\frac{k}{[OH^-]}\right)^2 - k_2\Sigma\frac{k}{[OH^-]} - k_3\Sigma k - Z\Sigma\frac{k^2}{[OH^-]}$$

or, as in the present work, by summing the squares of the individual E values derived from equation i) by the method previously described.

The residual variance of k_2, k_3 and Z can be obtained from the following equations:-

The variance of k_2 is given by

$$\frac{S^2\begin{vmatrix} \Sigma[OH^-]^2 & \Sigma k[OH^-] \\ \Sigma k[OH^-] & \Sigma k^2 \end{vmatrix}}{A} \quad \ldots\ldots vii).$$

The variance of k_3 is given by

$$\frac{S^2\begin{vmatrix} N & \Sigma k \\ \Sigma k & \Sigma k^2 \end{vmatrix}}{A} \quad \ldots\ldots viii).$$

The variance of Z is given by

$$\frac{S^2\begin{vmatrix} N & [OH^-] \\ [OH^-] & [OH^-]^2 \end{vmatrix}}{A} \quad \ldots\ldots ix).$$

Where A is the determinant

$$\begin{vmatrix} N & \Sigma[OH^-] & \Sigma k \\ \Sigma[OH^-] & \Sigma[OH^-]^2 & \Sigma k[OH^-] \\ \Sigma k & \Sigma k[OH^-] & \Sigma k^2 \end{vmatrix}$$

It is customary to express the accuracy of the values obtained in terms of the 95% confidence limit which in magnitude is given by $\pm 2 \times$ the standard error. For example the 95% confidence limit for, say k_2 is given by $k_2 = a \pm 2\sqrt{\text{variance}}$.

The above procedure was applied successfully to the data for the 6-tosyl esters of methyl α and β -D-glucoside and that of 1,5-anhydro -D-glucitol in all of which the value of $\frac{k}{[OH^-]}$ showed a marked upward trend with increasing concentrations of sodium hydroxide. However, the method cannot be applied to the data for methyl 6-O-tosyl- α and β -D-galactoside because of the small range of values of $\frac{k}{[OH^-]}$. As discussed earlier, the calculations for these compounds were based on assumed values for Z (2 or 3). For the calculations the rate equation was rewritten in the form

$$\frac{k}{[OH^-]} + Z\,k = k_2 + k_3[OH^-] + E.$$

The terms on the left hand side may be regarded as an imdependent variable and represented by the symbol r. The equation then becomes

$$r = k_2 + k_3[OH^-] + E.$$

The "normal" equations for the least squares method are then as follows:-

i) $$Nk_2 + \Sigma k_3[OH^-] = \Sigma r.$$

ii) $$\Sigma k_2[OH^-] + \Sigma k_3[OH^-]^2 = \Sigma r[OH^-].$$

The residual variance of r is again given by the expression $S^2 = \frac{\Sigma E^2}{N-P}$ where N,P and S are as previously defined and in this instance P = 2.

The variance of k_2 is given by

$$S^2 \frac{\Sigma[OH^-]^2}{\begin{vmatrix} N & \Sigma[OH^-] \\ \Sigma[OH^-] & \Sigma[OH^-]^2 \end{vmatrix}}$$

The variance of k_3 is given by

$$S^2 \frac{N}{\begin{vmatrix} N & \Sigma[OH^-] \\ \Sigma[OH^-] & \Sigma[OH^-]^2 \end{vmatrix}}$$

The arithmetic involved in all of the above calculations was done mechanically using a Remington Rand Printing Calculator. The values of k^G given in Part 11 c (Tables 11-15) were used, all of the replicate observations being included. The values obtained for k_2, k_3 and Z, and their 95% confidence limits are given in Table 17, P.88. By way of example, the main steps of the calculation for methyl 6-O-tosyl-α-D-glucoside are given below. The random nature of the errors (E) can be seen from the table; this justifies the mathematical assumptions made.

Methyl 6-O-Tosyl-α-D-glucoside

Computation of least squares for the equation $\frac{k}{[OH^-]} = k_2 + k_3 [OH^-] - Z\,k + E$

N	$[OH^-]$	$10^5 k$	$\frac{10^5 k}{[OH^-]}$	$[OH^-]^2$	$10^{10} k^2$	$10^5 k [OH^-]$	$\frac{10^{10} k^2}{[OH^-]}$	E	E^2
1	0.02	2.37	118.50	0.0004	5.6169	.0474	280.8450	-3.24	10.4986
1	0.02	2.27	113.50	0.0004	5.1529	.0454	257.6450	-8.47	71.7409
1	0.02	2.25	112.50	0.0004	5.0625	.0450	253.1250	-9.52	90.6304
1	0.05	7.93	158.60	0.0025	62.8849	.3965	1257.6980	+9.53	90.8209
1	0.05	7.56	151.20	0.0025	57.1536	.3780	1143.0720	+1.26	1.5876
1	0.05	7.98	159.60	0.0025	63.6804	.3990	1273.6080	+10.64	113.2096
1	0.05	7.42	148.40	0.0025	55.0564	.3710	1101.1280	-1.86	3.4596
1	0.1	19.20	192.00	0.01	368.64	1.9200	3686.4000	+2.04	4.1616
1	0.1	19.40	194.00	0.01	376.36	1.9400	3763.6000	+4.50	20.2500
1	0.1	19.50	195.00	0.01	380.25	1.9500	3802.5000	+5.74	32.9476
1	0.2	49.60	248.00	0.04	2460.16	9.9200	12300.8000	-5.43	29.4849
1	0.2	49.60	248.00	0.04	2460.16	9.9200	12300.8000	-5.43	29.4849
1	0.2	50.50	252.50	0.04	2550.25	10.1000	12751.2500	+1.17	1.3689
1	0.4	134.00	335.00	0.16	17956.0	53.6000	44890.0000	+9.62	92.5444
1	0.4	129.00	322.50	0.16	16641.0	51.6000	41602.5000	-14.53	211.1209
1	0.4	132.00	330.00	0.16	17424.0	52.8000	43560.0000	-0.04	0.0016
1	0.4	133.00	332.50	0.16	17689.0	53.2000	44222.5000	+4.79	22.9441
17	2.76	773.58	3611.80	.8012	78560.4276	248.6323	228444.471	-	826.256

The normal equations are:

1) $17k_2 + 2.76k_3 - 773.58Z - 3611.80 = 0$

2) $2.76k_2 + 0.8012k_3 - 248.6323Z - 773.58 = 0$

3) $773.58k_2 + 248.6323k_3$ [illegible]

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